Inhibition of Mutagen Formation by Organosulfur Compounds

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The effect of the organosulfur compounds, L-cysteine, L-cystine, reduced glutathione, deoxyalliin, and *N*-acetyl-L-cysteine, on mutagen formation in a meat model system was examined. Samples containing glucose, glycine, and creatinine were heated in a diethylene glycol reflux system at 150 °C in the presence or absence of organosulfur compound. The kinetics of brown color development appeared to be best described by biphasic zero-order kinetics whereas, mutagen formation was described by zero-order kinetics with plateau formation. Glutathione, L-cysteine, L-cystine, and deoxyalliin inhibited the formation of mutagens in the model system. However, although *N*-acetyl-L-cysteine inhibited color formation, it had no effect on mutagen formation. Similarly, L-cystine had no effect on browning but was a potent inhibitor of mutagen formation.

Keywords: *Maillard reaction; mutagenicity; organosulfur compounds; kinetics; L-cysteine; L-cystine; glutathione; deoxyalliin; N-acetyl-L-cysteine*

INTRODUCTION

Cooking or storage of foodstuffs that contain carbonyl and amino compounds result in nonenzymatic or Maillard browning reactions. A variety of products are formed that produce changes in color, flavor, functional properties, and nutritional value of the food (O'Brien and Morrissey, 1989).

Many of these changes are highly desirable as they enhance the organoleptic properties of food. However, in some cases, the Maillard reaction results in the formation of various mutagens, especially in muscle meat systems, where the presence of creatin(in)e leads to the formation of a class of heterocyclic amines called the aminoimidazoazaarenes (AIAs) (Jagerstad et al., 1990; Pearson et al., 1992). AIAs have been demonstrated to exhibit strong mutagenic activity in the Salmonella/microsome mutagenicity assay in strains sensitive to frameshift mutations in the presence of metabolic activation (Snyderwine, 1994). In addition, many of the AIAs have been shown to be multitarget carcinogens in animal models (Sugimura and Sato, 1983; Bogen, 1994) and recent reports have suggested an association between colon cancer and consumption of red meat (Willett, 1995; World Cancer Research Fund, 1997; COMA, 1998). In addition, there is epidemiological evidence that the severe cooking of red meat is associated with a higher risk of colorectal cancer (Schiffman and Felton, 1990). Although estimates of the possible contribution of AIAs to human cancer vary (Layton et al., 1995), there is evidence that humans with the fast acetylator phenotype may be at higher risk of developing colon cancer (Roberts-Thomson et al., 1996).

Since much of the human population is exposed to these mutagens via the diet, it is important not only to quantify the actual risk to humans but also to find ways to minimize the extent of mutagen formation during cooking. Inhibition of mutagen formation during cooking may also be of benefit in the context of occupational health for food preparers since relatively high levels of airborne mutagens may be present in the fumes from meat cooking (Thiebaud et al., 1995).

Several approaches to decreasing mutagen formation during cooking have been assessed. Measures have included attempts to remove some of the creatin(in)e and other precursors using a microwave pretreatment (Felton et al., 1992). Mutagen formation during cooking also appears to be decreased by the addition of soy protein concentrate (Wang et al., 1982), mono- or disaccharides (Skog and Jagerstad, 1990), selected amino acids (Jones and Weisburger, 1988), and antioxidants (Kato et al., 1996). More recently, marinating has been shown to decrease the overall mutagenicity of cooked meat, but to increase the level of MeIQx formed (Salmon et al., 1997; Felton and Knize, 1998).

Organosulfur compounds and, especially, the sulfur amino acids and their derivatives appear to be particularly effective in inhibiting nonenzymatic browning reactions (Friedman, 1996). Although the degree of browning does not generally correlate with mutagen formation (Laser Reutersward et al., 1987), inhibition of Maillard reaction pathways may be of special significance for the formation of AIAs because of the importance of the reaction in generation of heterocyclic substrates (e.g., pyrazines and pyridines) for mutagen formation. The reactivity of organosulfur compounds suggests that they could also act as site-specific reagents for modifying active sites on mutagenic compounds both before and after metabolic activation (Friedman, 1994).

To determine whether organosulfur compounds could inhibit the formation of heterocyclic amine mutagens, the present study used a model cooked meat system consisting of glucose, glycine, creatinine, and test compounds in diethylene glycol. Reflux model systems based on diethylene glycol have an advantage over other models in facilitating uniform heating at constant temperature and moisture content and at atmospheric pressure. The degree of browning and the mutagenicity

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of diluted aliquots of the reaction mixture was assessed. In addition, mutagenicity was determined on polyaromatic fractions of reaction mixtures, prepared using blue-cotton treatment, to ensure the absence of interfering substances.

The Ames test was selected to monitor mutagen formation in the present study because it avoids the disadvantage of specific chromatographic methods that might not detect the unforeseen formation of additional mutagenic compounds due to changes in reaction pathways. As a screening technique it offers the advantage of sensitivity over the chromatographic methods, and as it represents a biological end-point, it is a more meaningful starting point for assessing the toxicological significance of variations in chemical composition.

EXPERIMENTAL PROCEDURES

Materials. Deoxyalliin (*S*-allyl-L-cysteine) was prepared according to the method described by Iberl et al. (1990). Purity was assessed using thin-layer chromatography. 2-Amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline (IQ) was purchased from ICN Biomedicals Lt, Thame, Oxon, UK. Diethylene glycol, methanol, and ammonium hydroxide were obtained from BDH Lt, Poole, Dorset, UK. Dimethyl sulfoxide (DMSO) was purchased from Fisons, Loughborough, Leicestershire, UK. All other chemicals used were from Sigma-Aldrich Company Ltd, Poole, Dorset, UK.

Salmonella typhimurium strains TA98 and TA100 were kindly provided by B. N. Ames (University of California, Berkerley, CA).

Rat liver S9 fraction was obtained from the livers of male Fischer 344 rats (mean weight, 201 g) induced with i.p. injections of 80 mg/kg sodium phenobarbitone and 80 mg/kg β-naphthoflavone. The protein concentration was adjusted to 30 mg/mL and the S9 metabolizing mixture made up as follows: 1 mL S9 fraction; 1 mL of $MgCl_2$ (80mM); 1 mL of KCl (330mM); 1 mL of nicotinamide adenine dinucleotide phosphate (40mM); 1 mL of glucose 6-phosphate (50mM); and 5 mL of sodium phosphate buffer (0.2M; pH 7.4). S9 fractions were stored in a liquid nitrogen freezer prior to use. S9 metabolizing mixture was made up freshly and stored on ice until used.

Model Systems. The reaction was carried out in a diethylene glycol−water reflux model cooked meat system at 150 °C in a total volume of 150 mL. The system contained equimolar amounts (70 mM) of glycine (0.788 g) and creatinine (1.188 g), 35 mM glucose (0.946 g) and 5% distilled water (7.5 mL). Reactions were conducted in the presence or absence of selected organosulfur compounds (L-cysteine, glutathione, deoxyalliin, L-cystine, and *N*-acetyl-L-cysteine). The concentration of organosulfur compound was 20 mM. Samples (~2 mL) were taken at set timepoints (0, 5, 10, 15, 20, 30, 40, 45, 50, 60, 75, 90, 120, 150, and 180 min) and were immediately placed in a chest freezer (−20 °C) to stop the reaction. The control reaction was conducted in triplicate.

Degree of Browning. The degree of browning was estimated by measuring the absorbance at 490 nm using a Perkin-Elmer Lamda 5 UV/vis spectrophotometer. Samples were diluted, as appropriate, with distilled water to bring values within the range 0−1 absorbance units. Distilled water was used as blank.

Mutagenicity. The mutagenicity of the samples was measured using the Salmonella/Mammalian-Microsome Assay (Ames Test) as described by Ames et al. (1975) and modified by Maron and Ames (1983). Preliminary experiments using *S. typhimurium* TA100 produced only a weak response compared with strain TA98 and, consequently, the latter was chosen as the most appropriate test strain. Similarly, no mutagenicity was observed in the absence of metabolic activation and all assays were conducted in the presence of S9 mix. The following diagnostic tests were conducted prior to the study to confirm the integrity of the TA98 and TA100 strains: preexisting mutants, spontaneous mutants, number of viable cells, ampicillin-resistant viable cells, diameter of zone of growth inhibition in the presence of crystal violet and response to appropriate positive controls. Spontaneous revertants were in the acceptable range of 10−75 colonies per plate.

Test solutions (50 μL) were preincubated with 0.5 mL of S9 mix and 100 μL of overnight bacterial suspension ($2-3 \times 10^8$ cfu) for 20 min at 37 °C. Following preincubation, top agar (2 mL) was added, suspensions were mixed and immediately poured onto minimal agar plates. All samples were tested in triplicate and IQ was used as standard. After 72 h at 37 °C, revertant colonies were counted manually using a colony counter (Anderman and Company Ltd, Molesey, UK). Multiple dilutions were prepared for each sample using DMSO. Dilutions that resulted in at least double the spontaneous reversion rate and less than ~800 colonies per plate, were considered the most appropriate.

From the counts for the known concentrations of IQ, a standard curve was drawn using regression analysis with an intercept equal to zero, from which the equivalents of IQ per milliliter of sample were calculated. Such standardization allowed for comparison among different experiments.

Extraction. Mutagens were extracted from samples by the method of Hayatsu et al. (1983) using blue cotton (i.e., trisulfocopper phthalocyanine covalently bound to cotton) as adsorbent and ammoniacal methanol (methanol:ammonia solution, 36%; 50:1, v/v) as the eluent. Such extracts were designated as polyaromatic fractions.

The method was modified to overcome the following limitations: (1) The original procedure is designed for aqueous samples, whereas the present reaction mixtures consisted largely of diethylene glycol which, in preliminary experiments, prevented adsorption. The original procedure is designed for dilute samples of mutagens, whereas the present reaction mixtures contained relatively high mutagenic activity (up to 300 ng of IQ equivalents/mL).

The modified procedure was as follows: to 0.5 mL of sample was added 9.5 mL of distilled water, was added and the solution was adjusted to pH 9 by addition of 0.01 M NaOH. Blue cotton (50 mg) was added, and the mixture was left to equilibrate for 30 min. The blue cotton was then removed from the mixture, rinsed with distilled water, and the excess water was removed by wiping with a paper tissue. This procedure was repeated in triplicate for each sample. Adsorbed mutagens were eluted by adding 7.5 mL of ammoniacal methanol, and the mixture was left to equilibrate for 30 min. The eluate was pipetted out and placed in a round-bottomed flask. This procedure was repeated three times. The eluate was evaporated to dryness under reduced pressure at 35 °C, using a rotary evaporator (Rotavapor R110, Buchi Laboratories − Technik AG, Flawil, Switzerland). The residue was redissolved in 5 mL of DMSO.

Statistical Analysis. All calculations and statistical analyses were performed using Microsoft Excel Version 7. Reaction orders were determined as described previously (O'Brien, 1996). In the case of mutagenicity data, plateau points were omitted from data analysis. Reaction rate constants were calculated from the best-fit lines. For the analysis of mutagenicity data, rate constants were compared using the method described by Armitage and Berry (1987) following a simple test to ensure homogeneity of the variances (Mager, 1991). The slopes and their standard errors were calculated for the mutagenicity versus time curves for test and control samples (b_1 and b_2). Differences were tested by calculating $t = (b_1 - b_2)/\mathrm{SE}(b_1 - b_2)$ for $n_1 + n_2 - 4$ degrees of freedom (Armitage and Berry, 1987).

RESULTS AND DISCUSSION

Degree of Browning. Nonenzymatic browning in the heated meat model system was significantly inhibited in the presence of L-cysteine, *N*-acetyl-L-cysteine, or reduced glutathione (Figure 1). However, deoxyalliin or L-cystine had no effect on browning rate. These

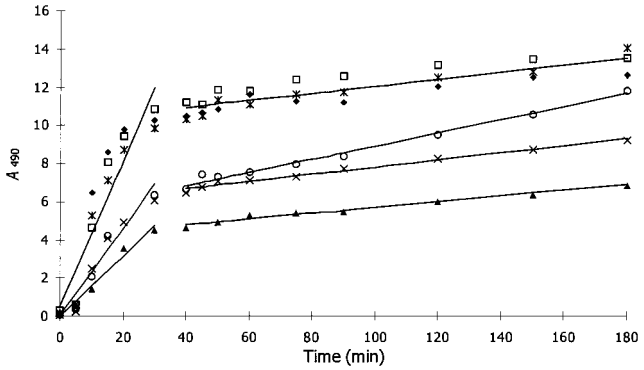

Figure 1. Nonenzymatic browning (A_{490}) of meat model systems in the absence (◆) or presence of 20 mM of selected organosulfur compounds: L-cystine (*); deoxyalliin (□); *N*-acetyl-L-cysteine (○); glutathione (×), and L-cysteine (▲)

Table 1. Coefficients of Determination (R^2) for Color Development of Heated Meat Model Systems

	R^2 by reaction order			
	0	1	2	sample size (n)
Simple Reaction (Time Range: 0−180 min)				
control	0.4555	0.2233	0.1023	15
L-cysteine	0.6965	0.4079	0.1765	13
N-acetyl-L-cysteine	0.7941	0.3955	0.1503	14
deoxyalliin	0.5256	0.3022	0.1740	15
glutathione	0.6609	0.3268	0.1552	15
L-cystine	0.5891	0.3012	0.1507	15
Biphasic Reaction (Initial Phase: 0−30 min)				
control	0.8218	0.6561	0.4024	6
L-cysteine	0.9814	0.8383	0.5037	5
N-acetyl-L-cysteine	0.9501	0.7525	0.4473	5
deoxyalliin	0.9078	0.7676	0.6079	6
glutathione	0.9338	0.7598	0.5572	6
L-cystine	0.8931	0.7328	0.5413	6
Biphasic Reaction (Final phase; 40−180 min)				
control	0.8856	0.8766	0.8666	9
L-cysteine	0.9779	0.9645	0.9440	8
N-acetyl-L-cysteine	0.9876	0.9847	0.9684	9
deoxyalliin	0.8941	0.8778	0.8601	9
glutathione	0.9819	0.9693	0.9516	9
L-cystine	0.9514	0.9436	0.9305	9

results are consistent with the previous studies which showed that *N*-acetyl-L-cysteine and glutathione could prevent nonenzymatic browning in model systems, fruit juices, and protein-containing foods at 100 °C (Molnar-Perl and Friedman, 1990; Friedman and Molnar-Perl, 1990).

Many studies on the kinetics of nonenzymatic browning have been published, but few have examined reactions at temperatures greater than 100 °C. Reactions are most frequently reported as being pseudo zero order with respect to color formation based on fitting data to zero-, first-, or second-order models (Baisier and Labuza, 1992). The most appropriate model is usually selected by comparison of coefficients of variation or correlation coefficients after omitting points corresponding to induction phases (O'Brien, 1996). However, we do not believe that such an approach is valid for the present data, as statistical analysis clearly indicates a biphasic reaction (Table 1). We have, therefore, reported separate rate constants for the two phases of the reaction (Table 2). This approach is further supported by the results of Arvidsson et al. (1997), which show the rapid depletion of the key reactant glucose in the early stages of such reactions, presumably leading to abrupt changes in reaction conditions. Color development appears to be rapid up to ~30 min, after which the reaction rate

Table 2. Rate Constants of Color Formation in Heated Meat Model Systems

	Rate Constant (k, Absorbance units·min^{-1})[a]	
system	initial phase, k_1	final phase, k_2
control	0.384 ± 0.248	0.015 ± 0.0047
L-cysteine	0.158 ± 0.031	0.015 ± 0.0019
N-acetyl-L-cysteine	0.235 ± 0.081	0.034 ± 0.0034
deoxyalliin	0.398 ± 0.176	0.018 ± 0.0054
glutathione	0.222 ± 0.082	0.019 ± 0.0023
L-cystine	0.359 ± 0.172	0.023 ± 0.0047

[a] Values ± 95% confidence intervals.

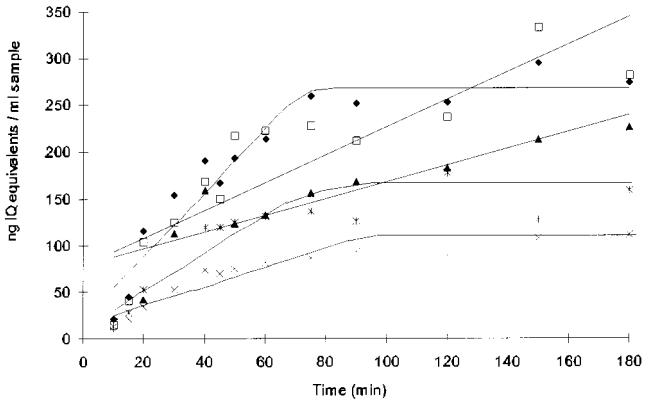

Figure 2. Mutagenicity (expressed as equivalents of IQ, ng/mL) of meat model systems heated in the absence (◆) or presence of 20 mM of selected organosulfur compounds: L-cystine (*); deoxyalliin (□); glutathione (×); and L-cysteine (▲)

decreases dramatically (up to 25-fold less than the initial rate). Both phases appear to fit pseudo-zero-order kinetics. A significant feature of the browning kinetics is that the final absorbance value appears to depend largely on the initial rate of browning as the k_2 values are similar in all cases except for the system containing *N*-acetyl-L-cysteine. To our knowledge, such behavior has not been reported for nonenzymatic browning at low temperatures and may be a consequence of the depletion of a key reactant or intermediate.

Suggested mechanisms of browning inhibition by sulfur compounds include free radical suppression and interaction with precursors of brown pigments, blocking the final steps in browning (Friedman and Molnar-Perl, 1990). Such mechanisms allow for the possible reaction of both free sulfydryl groups and free amino groups with reactants or intermediates in Maillard reaction pathways. However, the present data support the hypothesis of Friedman and Molnar-Perl (1990) that the inhibition of browning by organic sulfur compounds depends on a free sulfhydryl group. Thus, compounds without a free sulfhydryl group did not inhibit browning in the present study, whereas *N*-acetylcysteine, which had a free sulfhydryl group but a blocked amino group, was inhibitory.

Mutagenicity. The mutagenicity of model systems containing glutathione, deoxyalliin, L-cysteine, or L-cystine was significantly lower than the control system (Figure 2). The presence of *N*-acetyl-L-cysteine appeared to have no effect on the formation of mutagens.

Paradoxically, *N*-acetyl-L-cysteine had no effect on mutagen formation, whereas it inhibited the nonenzymatic browning reaction. Similarly, L-cystine had no effect on nonenzymatic browning kinetics but appeared to be a potent inhibitor of mutagen formation. These

results suggest that the mechanism of inhibition of nonenzymatic browning by organosulfur compounds is different from the mechanism of inhibition of mutagen formation. This implies that inhibition of nonenzymatic browning, per se, may not be a good selection criterion for inhibitors of mutagen formation, even though precursors of mutagen formation are produced in the browning pathways. Although a free sulfhydryl group appeared to be necessary for the inhibition of browning, there was no clear pattern in the structure of compounds that produced a decrease in mutagen formation. The fact that mutagen formation was not affected by *N*-acetyl-L-cysteine may point to the involvement of a reactive amino group in the mechanism of inhibition but clearly further work is necessary to study mechanisms. If the mechanism of inhibition of mutagen formation requires a reactive amino group, the effect of the organosulfur compounds studied may be similar to that of some other amino acids such as proline or tryptophan (Jones and Weisburger, 1988).

Rate constant data suggest that the potency of compounds in reducing mutagen formation was in the order: L-cysteine > glutathione > deoxyalliin > L-cystine. However, this ranking differs somewhat from a ranking based on final mutagenicity of heated samples which was as follows: glutathione > L-cystine > L-cysteine > deoxyalliin.

There is much evidence in the literature of the protective effect of many organosulfur compounds against the harmful biological effects of a range of electrophiles, including mutagens. There is evidence that deoxyalliin and possibly other organosulfur compounds from garlic may inhibit experimental colon and mammary carcinogenesis (Sumiyoshi and Wargovich, 1990; Amagase et al., 1996). Similarly, *N*-acetylcysteine has been reported to protect against the cytotoxicity of metabolites of IQ and PhIP (Davis and Snyderwine, 1995). Such activity of organosulfur compounds may be a function of their antioxidant activity (Stamler and Slivka, 1996), conversion to glutathione (Stamler and Slivka, 1996), or direct interaction with mutagens resulting in inactivation (Friedman, 1994).

It was therefore considered that the presence of unreacted organosulfur compounds in the reaction mixtures could have resulted in a decrease in mutagenicity by either decreasing metabolic activation by the S9 mixture or by blocking the biological activity of formed mutagens. Thus, the polyaromatic fraction containing the mutagens was separated from other constituents of the reaction mixtures to evaluate whether the decrease in mutagenicity was a consequence of decreased mutagen formation or secondary to inference with the Ames assay. The results of the mutagenicity analysis (Figure 3) clearly show that the pattern of mutagenicity of the polyaromatic fractions of the heated model systems is similar to that of the unfractionated samples. This suggests that the decrease in mutagenicity was a consequence of decreased overall mutagen formation in the reaction mixtures. The data also imply that the ability of organosulfur compounds to protect against the cellular damage caused by mutagens appears to be largely destroyed by heating at high temperatures.

The kinetics of mutagen formation differ somewhat from those of brown color development. There is clear evidence of plateau formation after prolonged heating. On the basis of goodness-of-fit, the most appropriate

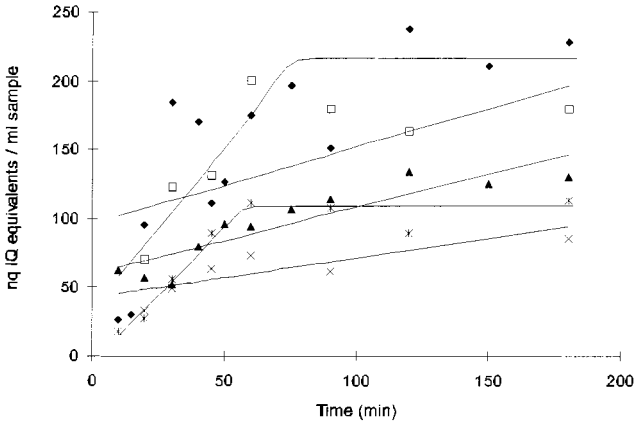

Figure 3. Mutagenicity (expressed as equivalents of IQ, ng/mL) of polyaromatic fractions of meat model systems heated in the absence (◆) or presence of 20 mM of selected organosulfur compounds: L-cystine (*); deoxyalliin (□); glutathione (×); and L-cysteine (▲).

Table 3. Coefficients of Determination (R^2) for Mutagen Formation in Heated Meat Model Systems

system	R^2 by reaction order 0	1	2	sample size (n)	time range (min)
control	0.8939	0.6944	0.4784	9	10−75
L-cysteine	0.7885	0.5629	0.3626	10	20−180
N−acetyl-L-cysteine	0.8926	0.715	0.4987	8	10−90
deoxyalliin	0.7514	0.4797	0.2282	13	10−180
glutathione	0.8532	0.6456	0.3938	10	10−90
L-cystine	0.8653	0.7593	0.5717	8	10−75

Table 4. Rate Constants of Mutagen Formation in Heated Meat Model Systems

system	k (ng of IQ equivalents mL^{-1} min^{-1})[a,b]
control	3.397 ± 1.046
L-cysteine	0.888 ± 0.375[a]
N−acetyl-L-cysteine	4.354 ± 1.509
deoxyalliin	1.482 ± 0.566[b]
glutathione	1.039 ± 0.351[a]
L-cystine	2.060 ± 0.812[a]

[a] Values ± 95% confidence interval. [b] Different letters indicate significant differences from control valve: a, $p < 0.001$; b, $p < 0.01$.

order appeared to be zero order after omission of the plateau points (Tables 3 and 4). To our knowledge, the only other study of kinetics of mutagen formation during cooking was that of Arvidson et al. (1997), who used a first-order model. In agreement with our observations, they also showed the development of plateau after prolonged heating.

Pearson et al. (1992) suggested that sugar fragmentation and free radical formation contribute to the formation of AIAs and suggested that antioxidants may inhibit mutagen formation by quenching free radicals. The findings of Kato et al. (1996) supported this hypothesis, by showing, using ESR, that the level of pyrazine cation radicals in a model system was decreased in the presence of BHA, sesamol or epigallocatechin gallate. It is possible that organosulfur compounds may act in a similar way.

However, sulfur amino acids may also participate directly in Maillard reactions. Such reactions may be very important in meat flavor (Mottram and Whitfield, 1995), and alliin and deoxyalliin have been shown to react with glucose in a Maillard reaction (Yu et al., 1994). Such reactions may compete with pathways that result in mutagen formation.

The present results suggest that selected organosulfur compounds may be useful inhibitors of mutagen formation during the cooking of meat. In addition, the tissue antioxidant glutathione appeared to be the most potent inhibitor of mutagen formation of the selected compounds. This may be of toxicological significance as tissue glutathione levels may vary considerably among different cuts of meat, different species, and different animals (Faustman and Cassens, 1991). Animals suffering oxidative stress premortem may have reduced tissue glutathione levels. The presence of high glutathione and glutathione peroxidase levels in red muscle fibers compared with other tissues has been reported for several species and appears to be primarily involved in defense against the effects of oxidation reactions (Chan and Decker, 1994). In beef, the gluteous medius muscle appears to have lower glutathione levels than the longissimus muscle (Faustman and Cassens, 1991). In general, freshly prepared meats are among the richest dietary sources of glutathione, but levels appear to decrease on storage (Faustman and Cassens, 1991; Jones et al., 1992). However, the inhibition of mutagen formation by L-cystine in the present study suggests that inhibition of mutagen formation might be independent of oxidation state of the S atom. Thus, oxidized glutathione might also be inhibitory. The present results suggest that the potential of meat composition to influence mutagen formation requires further study.

Recent work suggests that while marinating led to a dramatic reduction in the levels of PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*f*]pyridine) in cooked chicken, the levels of MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline) were actually increased (Salmon et al., 1997). The total detectable heterocyclic amine levels were decreased by up to 30-fold depending on cooking time. Marinating might lead to decreased mutagen formation by several potential mechanisms: extraction/dilution of precursors of mutagen formation (e.g. creatin(in)e); added sugar, as used in marinades, can decrease mutagen formation (Skog and Jagerstad, 1990; Salmon et al., 1997); the decrease in pH caused by acidic components of marinades can modify Maillard chemistry, decreasing mutagen formation; added flavor constituents of marinades, such as organosulfur constituents from *Allium* spp, could lead to a further reduction in mutagen formation by reacting with precursors or intermediates.

Marinating appeared to decrease the mutagenicity of samples cooked for moderate periods, whereas prolonged heating produced higher mutagenicity in the marinated samples than in controls (Salmon et al., 1997). Such findings emphasize the importance of examining the kinetics of mutagen formation rather than basing conclusions on single samples and also highlight the need to follow up studies of mutagenicity by deriving profiles of the mutagens formed on cooking. Since the carcinogenic potency of the AIAs appears to be variable, such data would be of value in assessing the benefits of inhibiting mutagen formation.

ABBREVIATIONS USED

AIAs, Aminoimidazo azarenes; IQ, 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline; DMSO, dimethyl sulfoxide; MeIQx, 2-amino-3,4(or 8)-dimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine.

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