PROBABLE MECHANISM(S) INVOLVED IN MEAT MUTAGEN FORMATION AND INHIBITION

A. M. PEARSON,[a,b] C. CHEN,[a,c] J. I. GRAY and S. D. AUST[d] Departments of Food Science and Human Nutrition[a] and Biochemistry[b] Michigan State University, East Lansing, MI 48824 U.S.A.

SUMMARY:

Amadori rearrangement is a key step in the Maillard reaction and results in sugar fragmentation and free radical formation. We propose that imidazoquinoline meat mutagens (2-amino-3-methylimidazo[4,5-f]quinoline or IQ and 2-amino-3,4- dimethylimidazo[4,5-f]quinoline or MeIQ) are produced by a reaction mixture of alkyl-pyridine free radicals and creatinine. On the other hand, imidazoquinoxaline meat mutagens (2-amino-3,4-dimethylimidazo[4,5-f]quinoxaline or MeIQx and 2-amino-3,4,8-trimethylimidazo[4,5-f]-quinoxaline or 4,8-DiMeIQx) are formed by reacting dialkylpyrazine free radicals and creatinine. We propose two different pathways for free radical formation. One involves bimolecular ring formation from the enaminol form of the glycoaldehyde alkylimine followed by oxidative formation of the free radical. The other pathway involves formation of N,N1-dialkylpyrazinium ions from glyoxal monoalkylimine followed by reduction to produce the free radicals. The glycoaldehyde system reacts faster and produces more free radicals than the glyoxal system, which helps to explain why imidazoquinoxaline meat mutagens are present in larger quantities in fried ground beef than the imidazoquinoline type meat mutagens. We also explain how BHT enhances mutagenicity and the pathways by which free radical scavenger type antioxidants, such as BHA, PG and TBHQ, and sulfiting agents and nitrite inhibit mutagenicity.

INTRODUCTION

Mutagens formed during cooking and/or processing of muscle foods have been divided into two groups by Miller (1985), namely, those produced by pyrolysis of proteins by heating in excess of 300°C, and those formed at moderate temperatures (<300°C). Most of the high temperature-induced mutagens are protein pyrolysates according to Sugimura (1986) and are 2-amino-pyridine-type mutagens (Furihata et al., 1986). The moderate temperature-induced mutagens are 2-amino-imidazole-type compounds (Furihata et al., 1986) and contribute most of the mutagenic activity found in cooked meat and fish (Kasai et.al., 1979, Nagao et al., 1981; Jagerstad et al., 1983; Felton et al., 1986a,b). Jagerstad et al. (1983a,b, 1984, 1986) and Grivas et al. (1985, 1986) have proposed that most of the moderate temperature-induced mutagens are produced from creatinine, aldehydes and Maillard reaction products, although the mechanisms involved in their formation are largely unknown.

IQ and MeIQ are imidazoquinoline compounds, while MeIQx, 4,8-DiMeIQx and 7,8-DiMeIQx are imidazoquinoxaline mutagens (Furihata et al., 1986; Jagerstadt et al., 1984; Grivas et al., 1985). Two other mutagens produced at moderate temperatures during cooking of meat are 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP) and 2-amino-N,N,N-trimethylimidazopyridine (TMIP), which are imidazopyridines. Both groups of compounds have been identified in cooked fish (Kasai et al., 1979; Nagao et al., 1981; Kikigawa and Kato, 1987,) and meat (Felton et al., 1986b; Jagerstad et al., 1983c

Yoshida and Okamoto (1980) demonstrated that heating of creatine or of amino acids with glucose produced mutagenic activity and suggested all conditions required to produce such activity are present during cooking of

Nevertheless, the mechanism(s) by which the Maillard reaction and free radicals result in the formation of mutagens during cooking/processing of meat are not clear. Therefore, we propose a pathway for mutagen production and also attempt to explain how BHT enhances mutagenicity by accelerating formation of the precursors for 4,8-DiMeIQx. Consideration is also given to the mechanisms by which nitrite, sulfites and other food additives may block formation of imidazoquinoline and imidazoquinoxaline type mutagens.

FREE RADICALS RESULT IN FORMATION OF IMIDAZOQUINOLINE AND IMIDAZOQUINOXALINE MUTAGENS:

Hodge (1953) proposed that Amadori rearrangement is a key step in the Maillard reaction. More recently, Namaki and Hayashi (1983) proposed that the Maillard reaction involves sugar fragmentation with free radical formation. Jagerstad et al. (1983b, 1984, 1986) and Grivas et al. demonstrated that IQ, MeIQx and DiMeIQx are produced by heating a model system containing creatinine with glycine or alanine and fructose by a free radical reaction. The exact pathways by which free radicals play a role in formation of imidazoquinoxalines and imidazo- quinolines have not been elucidated, but the mechanisms proposed appear to be logical. The basic concept that IQ and MeIQ are formed from a reaction mixture containing alkylpyridine free radicals and creatinine is consistent with model system studies (Jagerstad et al., 1984, 1986; Grivas et al., 1985, 1986).

The mechanism involves formation of an isolated two-carbon fragment to produce a glyoxal diimine derivative. The initial two-carbon fragment may be a glycosylamino compound produced by a reverse-aldol-type reaction. The two-carbon fragment can be oxidized easily to produce a glyoxal monoimine derivative, that would then form the glyoxal diimine derivative, which has been isolated from the reaction mixture previously by Namiki and Hayashi (1983). Although the glycolaldehyde alkylimines initially may be produced as fragmentation products, they may be readily oxidized to form the glyoxal monoalkylimine and give rise to the glyoxal dialkylimine.

There are two different pathways by which formation of free radicals may be produced by the Maillard reaction. The first is by biomolecular ring formation to produce the enaminol form of the glycolaldehyde alkylimine, which then by means of oxidation forms the free radical product. The second pathway occurs by formation of N,N^1-dialkylpyrazinium ions from glyoxal monoalkylimine and is followed by reduction to yield the free radicals. The intermediates for these pathways, glycolaldehyde alkylimine and glyoxal monoalkylimine, respectively, are formed by the reaction of glycolaldehyde or glyoxal with amino compounds. The glycolaldehyde system has been reported to react much faster and to produce a greater amount of free radicals than the glyoxal system (Namaki and Hayashi, 1983). Thus, the glycolaldehyde system is the predominant intermediate and helps in explaining why imidazoquinoxaline meat mutagens (MeIQx and 4,8-DiMeIQx) are the major mutagens formed during frying of fish (Kikugawa and Kato, 1987) and beef (Knize et al., 1987). Formation of the glyoxal dialkylimine is readily reversed under acid conditions to yield the glyoxal monoalkylimine derivative. Subsequent reduction will produce glycolaldehyde monoalkylimine, which is the precursor for free radical (dial- kylpyrazine free radicals) formation.

Although water (Overik et al., 1989) and sugar may not always be necessary for development of the meat mutagens, creatinine (creatine) and free amino acids are necessary (Overik et al., 1989) for their formation and may be involved in production of free radicals. Namiki and Hayashi (1975) observed that the primary and secondary structures of the compounds formed in the Maillard reaction depend on the specific amino acid used in

the mixture. Namiki and Hayashi (1983) have shown that both N,N^1-dialkylpyrazinium salt and a mixture of glycolaldehyde and an amino compound are highly active in forming free radicals. Support is found in the fact that free radical scavenger-type antioxidants, such as BHA, PG and TBHQ, inhibit formation of these mutagens (Chen 1988). It is suggested that the free radical scavenger type antioxidants stabilize the sugar fragment or else react with the free radicals formed by the Maillard reaction (either with the alkylpyridine free radicals or dialkylpyrazine free radicals) and thus, directly inhibit formation of the meat mutagen precursors.

HOW BHT ENHANCES FORMATION OF MEAT MUTAGENS:

BHT has been shown to enhance the total mutagenic activity of the meat mutagens (Chen, 1988). BHT can act as an alkylating agent and increase the formation of the precursors for 4,8-DiMeIQx. The methyl group, probably at the para-position of the BHT molecule, reacts with the dialkylpyrazine free radical and creatinine to become the methyl group at the 8-position and then forms 4,8-DiMeIQx. The reason that BHA does not behave like BHT is because the methoxy group on BHA becomes a quinone-like compound and is a potent free radical scaven- ger in contrast to the methyl group from the BHT molecule. The quinone-like compound formed by the methoxy group of BHA blocks the reaction before it can occur. TBHQ and PG inhibit meat mutagen formation in the same way as BHA since all of these compounds have more than one hydroxy group attached to their aromatic rings.

BLOCKING MEAT MUTAGEN FORMATION WITH TOCOPHEROLS AND SULFITING AGENTS:

Tocopherols are the best known natural antioxidants and also serve as free radical scavengers (Dugan, 1981). Inhibition of IQ-like compounds can take place in two ways: (1) It may block free radical formation directly, or (2) Break-down products of tocopherols may react with precursors of the meat mutagens and prevent formation of 4,8-DiMeIQx (Chen, 1988).

Sulfiting agents are well known as inhibitors of the Maillard reaction. Sulfites react readily with a variety of food constituents including reducing sugars, aldehydes, ketones and proteins to produce various sulfite combinations that block the Maillard reaction and production of free radicals.

INHIBITION OF MUTAGEN FORMATION BY NITRITE:

Chen (1988) showed that nitrite has a strong inhibitory effect on meat mutagen production. Kanner (1979) has shown that nitrite reacts with cysteine to produce S-nitrosocysteine, which was demonstrated to be a strong antioxidant, presumably by blocking free radical production. Preferential reaction of nitrite with cysteine in the meat system could result in production of N-nitrosocysteine and block mutagen production during cooking/ processing of meat.

CONCLUSIONS:

Free radicals can be produced in the Maillard reaction by two pathways. One is through bimolecular ring formation from the enaminol form of the glycolaldehyde alkylimine, which is followed by oxidative formation of the free radical. The other pathway involves formation of N,N^1-dialkylpyrazinium ions from glyoxal alkylimine, which is followed by reduction to yield free radicals. The glycolaldehyde system appears to be the preferred pathway as it is the predominant intermediate. This explains the formation of the imidazoquinoxaline type meat mutagens (MeIQx and 4,8-DiMeIQx), which are the major mutagens formed on frying of fish or beef.

BHT enhances mutagenicity by providing the methyl group that reacts with alkylpyrazine free radicals and creatinine to produce 4,8-DiMeIQx. The other synthetic antioxidants (BHA, PG and TBHQ) inhibit mutagenicity

because they contain methoxy groups and serve as potent free radical scavengers to block formation of the meat mutagen precursors. The tocopherols and sulfites also block mutagen development by preventing the Maillard reaction and free radical formation. Nitrite reacts with cysteine to produce N-nitrosocysteine, that can block free radical production and prevent formation of meat mutagens. Figures will be used to illustrate how formation and inhibition of mutagens can be achieved.

Acknowledgements - This report was made possible by a grant-in-aid from the National Live Stock and Meat Board, Chicago, Illinois, and by funds from the Michigan Agricultural Experiment Station.

REFERENCES:

Chen, C. (1988): Factors influencing mutagen formation during frying of ground beef.Ph.D thesis Michigan State University, East Lansing, Michigan

Dugan, L.R. Natural antioxidants. In: (Simic, M.G.; Karel, M., eds). "Autoxidation in Food and Biological Systems. Plenum Press, New York. pp. 261-282; 1981.

Felton, J.S., Knize, M.G., Shen, N.H., Lewis, P.R., Andresen, B.D., Happe, J.A., Hatch, F. T. (1986a): The isolation and identification of a new mutagen from fried ground beef: 2-amino-1-methyl-6- phenylimidazo -[4,5-b]pyridine (PhIP). Carcinogenesis 7: 1081-1086.

Felton, J.S., Knize, M.G., Shen, N.H., Andresen, B.D., Bjeldanes, L.F. and Hatch, F.T. (1986b). Identification of the meat mutagens in cooked beef. Environ. Health Perspect. 67: 17-24.

Furihata, C.and Matsushima, T. (1986): Mutagens and carcinogens in foods. Ann. Rev. Nutr. 6, 67-94.

Grivas, S., Nyhammar, T., Olsson, K. and Jagerstad, M. (1985): Formation of a new mutagenic DiMeIQx compound in a model system by heating creatinine, alanine and fructose. Mutat. Res. 151: 177-183.

Grivas, S., Nyhammar, T., Olsson, K. and Jagerstad, M. (1986): Isolation and identification of the food mutagens IQ and MeIQx from a heated model system of creatinine, glycine and fructose. Food Chem. 20: 127-136.

Hodge, J.E.(1953):Dehydrated foods.Chemistry of browning reaction in model systems. J.Agric. Food Chem.1:928-943.

Jagerstad, M., Laser Reutersward, A., Oste, R., Dahlquist, A., Grivas, S., Olsson, K. and Nyhammar, T.(1983 a):Creatinine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In: Maillard reactions in foods and nutrition. ACS Symposium Series 215:Waller, G.R. Feather,M.S.eds.507-519.

Jagerstad, M., Laser Reutersward, A.,Olson, R.,Grivas, S.,Nyhammar, T.,Olsson,K. and Dahlquist,A.(1983 b): Creatin(in)e and Maillard reaction products as precursors of mutagenic compounds: Effect of various amino acids. Food Chem. 12: 255-264.

Jagerstad, M., Olsson, K., Grivas, S., Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S. and Sugimura, T.(1984): Formation of 2-amino-3,8-dimethylimidzo-[4,5-f]-quinoxaline in a system by heating creatinine, glycine and glucose. Mutat. Res. 126: 239-244.

Jagerstad, M., Grivas, S., Olsson, K., Laser Reutersward, A., Negishi, C. and Sato, S. (1986): Formation of food mutagens via Maillard reactions. Prog. Clin. Biol. Res. 206: 155-167.

Kanner, J. (1979):S-Nitrosocysteine (RSNO), an effective antioxidant in cured meat.J.Am.Oil Chem.Soc.56:74-76.

Kasai, H., Nishimura, S., Nagao, M., Takahashi, Y. and Sugimura, T. (1979): Fractionation of a mutagenic principle from broiled fish by high-pressure liquid chromatography. Canc. Lett. 7: 343-352.

Kikugawa, K. and Kato, K.(1987):Formation of mutagens, 2-amino-3, 8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx) in heated fish meats. Mutat.Res.179:5-14.

Knize, M.G., Happe, J.A., Healy, S.K. and Felton, J.S. (1987): Identification of the mutagenic quinoxaline isomers from fried ground beef. Mutat. Res. 178: 25-32.

Miller, A.J. (1985): Processing-induced mutagens in muscle foods. Food Technol. 39 (2): 75-79.

Nagao, M., Wakabayashi, K., Kasai, H.; Nishimura, S. and Sigimura, T. (1981): Effect of methyl substitution on mutagenicities of 2-amino-3-methylimidazo[4,5 -f] quinoline isolated from broiled sardine. Carcinogenesis 2: 1147-1156.

Namiki, M. and Hayashi, T. (1983): A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. In: "The Maillard reaction in foods and nutrition ACS Symposium Series 215:(Waller, G.R. and Feather, M.S., eds.) 21-46.

Sugimura, T. (1986) Studies on environmental chemical carcinogenesis in Japan. Science 233: 312-315.

Yoshida, D. and Okamoto, H.(1980):Formation of mutagens by heating creatine and glucose. ppBiochem.Biophys.Res. Commun. 96, 844-847.