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Past, Present, and Future of Mutagens in Cooked Foods

by Takashi Sugimura*

Mutation assay with *Salmonella typhimurium* enabled us to detect various types of mutagens in cooked foods. A series of mutagenic heterocyclic amines has been isolated and identified in broiled fish and meat and in pyrolyzates of amino acids and proteins. Feeding experiments showed these mutagens to be carcinogenic in mice and rats. The mechanism of formation and pathway of metabolic activation of these heterocyclic amines have been elucidated. Their contents in various cooked foods have been determined. The presence of mutagenic nitropyrenes (some of which were confirmed as carcinogens) in grilled chicken was also established. Roasted coffee beans also yield mutagens such as methylglyoxal. The formation of mutagen precursors, including β-carboline derivatives and tyramine which become mutagens with nitrite treatment, was found during food processing. Oncogene activation in animal tumors induced by some of these food mutagens/carcinogens has been confirmed. The role of mutagens/carcinogens in cooked foods in human cancer development has not yet been exactly evaluated. In order to do this, more information on their carcinogenic potency, human intake, metabolism in the human body, and the effects of combined administration with other initiators, promoters and other modifying factors in food is required.

General Background

It has been generally accepted that the incidence of cancers in different organs varies very much from nation to nation. Studies on immigrants also revealed that different cancer incidence in various areas of the world depends on the life style, including dietary habits. Careful epidemiological studies led us to conclude that the dietary factor was one of the most important causes of human cancers, as important as cigarette smoking (*1–3*).

Diet influences the incidence of cancer in many ways, directly and indirectly. In one field of research, trials to detect known carcinogens in food were made many years ago. These are described in two papers by Kuratsune (*4*) and by Lijinsky and Shubik (*5*) on the detection of carcinogenic aromatic hydrocarbons in biscuits and broiled meat. Carcinogenesis experiments are time consuming and require many animals. Therefore it was very difficult to search for new carcinogens other than those already known, such as aromatic hydrocarbons, mycotoxins and dialkylnitrosamines from foods.

Since the overlapping of mutagens and carcinogens was suggested, especially from data obtained by using the Ames test with *Salmonella typhimurium* (*6–8*), it became possible to predict the presence of carcinogens in food through the detection of mutagenic activity of food. The formation of mutagens detectable by the Ames test upon broiling dried fish and ground meat was reported by us (*9*) and Commoner et al. (*10*). These studies prompted us to identify mutagens formed during cooking. New mutagens have been discovered successively and these chemicals synthesized and subjected to long-term animal tests. Some of them have now been demonstrated to be carcinogenic in mice and rats (*11–15*). Thus, the introduction of microbial mutation to screen for carcinogens formed by cooking was proven to be practical and realistic.

Mutagens Found in Cooked Foods and Heated Amino Acids and a Protein

Kasai et al. (*16,17*) were the first to isolate and identify aminoimidazoquinoline (IQ) and aminomethylimidazoquinoline (MeIQ) from broiled sun-dried sardines (Table 1). They (*18*) also isolated aminomethylimidazoquinoxaline (MeIQx) from fried beef. The contributions of the Maillard reaction and of the presence of creatinine in meat to the formation of aminoimidazoquinolines and aminomethylimidazoquinoxalines, were proposed (*19*). Actually, the formation of MeIQx and DiMeIQx after heating of the mixtures of creatinine, sugars and amino acids was demonstrated by Jägerstad et al. (*20*) and Negishi et al. (*21,22*). The specific mutagenic activities of IQ, MeIQ, and MeIQx are very high towards *S. typhimurium* TA 98 with S–9 mix as shown in Table 2. Ohgaki et al. demonstrated the carcinogenicity of IQ (*23*) and MeIQ (*24*), in mice while Takayama et al. (*25*) and Tanaka et al. (*26*) proved the carcinogenicity of IQ in rats. The locations of tumors in mice

*National Cancer Center, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan.

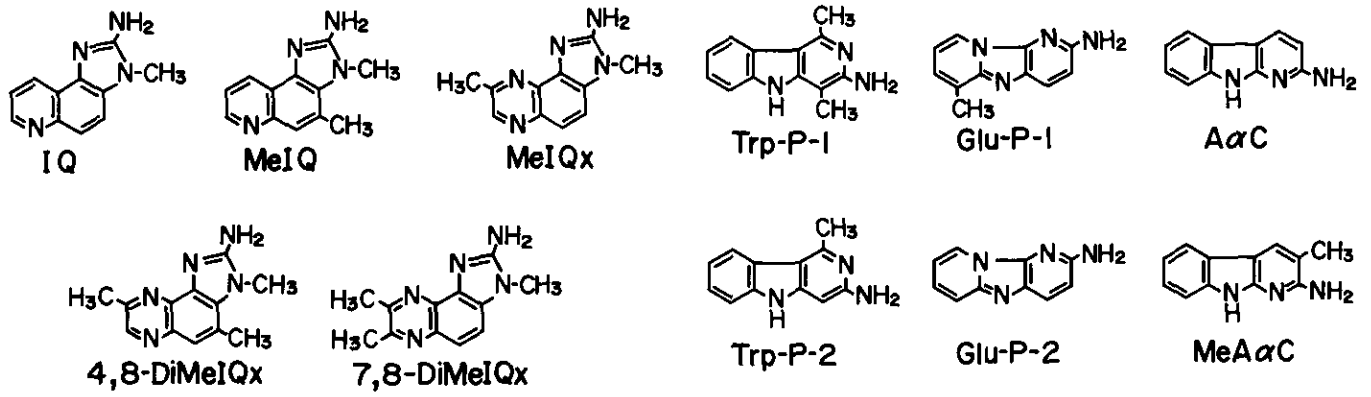

Table 1. Abbreviations used.

Abbreviation	Compound
IQ	2-Amino-3-methylimidazo[4,5-*f*]quinoline
MeIQ	2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline
MeIQx	2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline
4,8-DiMeIQx	2-Amino-3,4,8-trimethylimidazo[4,5-*f*]-quinoxaline
7,8-DiMeIQx	2-Amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline
Trp-P-1	3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole
Trp-P-2	3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole
Glu-P-1	2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]-imidazole
Glu-P-2	2-Aminodipyrido[1,2-*a*:3′2′-*d*]imidazole
AαC	2-Amino-9*H*-pyrido[2,3-*b*] indole or 2-amino-α-carboline
MeAαC	2-Amino-3-methyl-9*H*-pyrido[2,3-*b*]indole or 2-amino-3-methyl-α-carboline

Table 2. Mutagenic activities of aminoimidazoquinolines and aminoimidazoquinoxalines toward *S. typhimurium* with S-9 mix.

	Mutagenic activity, revertants/μg	
	TA 98	TA 100
IQ	433,000	7,000
MeIQ	661,000	30,000
MeIQx	145,000	14,000
4,8-DiMeIQx	183,000	8,000
7,8-DiMeIQx	163,000	9,900

Table 3. Location of tumors induced by IQ and MeIQ in mice and rats.

	Mouse	Rat
IQ	Liver Forestomach Lung	Liver Small and large intestines Zymbal gland Clitoral gland Skin Oral cavity
MeIQ	Liver Forestomach Small and large intestines	Not done

and rats are given in Table 3. The metabolic conversion of the amino group of these heterocyclic amines to the hydroxyamino group catalyzed by cytochrome P-450s is essential to the exertion of their mutagenic activities.

Some heterocyclic amines are isolated from pyrolyzates of amino acids and a protein as a model system of charred food. Thus, Trp-P-1 and Trp-P-2 from a tryptophan pyrolyzate were reported by Sugimura et al. (*27*) and Glu-P-1 and Glu-P-2 from a glutamic acid pyrolyzate were reported by Yamamoto et al. (*28*). Amino-α-carboline (AαC) and methylamino-α-carboline (MeAαC) were isolated from a pyrolyzate of soybean globulin by Yoshida et al. (*29*). The presence of Glu-P-2 (*30*), Trp-P-1 and Trp-P-2 (*31*), and AαC and MeAαC in broiled foods (*32*) and of AαC and MeAαC in cigarette smoke condensates (*32,33*) has been reported. These heterocyclic amines also possess a fairly high mutagenic activity toward *S. typhimurium* TA 98 with S9 mix as shown in Table 3. Trp-P-1 and Trp-P-2 were demonstrated to be carcinogenic in mice by Matsukura et al. (*34*) and in rats by Hosaka et al. (*35*) and Takayama et al. (*36*). Carcinogenicity of Glu-P-1 and Glu-P-2 was described in mice by Ohgaki et al. (*37*) and in rats by Takayama et al. (*38*). AαC and MeAαC were carcinogenic in mice (*37*), but experiments on rats are being repeated due to less carcinogenic potential of AαC and greater toxic potential of MeAαC than shown in mice; in rats MeAαC induces atrophy of the salivary glands and pancreatic acinar cells, as observed by Takayama et al. (*39*). The locations of tumors in mice and rats fed on diets containing these heterocyclic amines are listed in Table 5.

These heterocyclic amines are metabolized to *N*-hydroxyamino derivatives, and acetyl or sulfate esters would be the ultimate forms which react with DNA as shown by Yamazoe et al. (*40,41*), Hashimoto et al. (*42,43*), and Nagao et al. (*44*).

Tsuda et al. found that Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, AαC, and MeAαC were sensitive to nitrite treatment, while IQ, MeIQ, MeIQx, and DiMeIQx are resistant, and that both groups of heterocyclic amines lost their mutagenic activity after treatment with hypochlorite (*45–47*). Differential inactivation of mutagenic activity by these treatments enabled us to estimate the contributions of IQ-type and non-IQ-type

Table 4. Mutagenic activities of heterocyclic amines isolated from pyrolyzates of amino acids and a protein toward *S. typhimurium* with S-9 mix.

	Mutagenic activity, revertants/μg	
	TA 98	TA 100
Trp-P-1	39,000	1,700
Trp-P-2	104,200	1,800
Glu-P-1	49,000	3,200
Glu-P-2	1,900	1,200
AαC	300	20
MeAαC	200	120

heterocyclic amines to the total mutagenicity in basic fractions from various materials. For instance, the mutagenic activity of charred parts of broiled beef consists mainly of IQ-type heterocyclic amines but that of cigarette smoke condensate consists mainly of non-IQ-type mutagens (*15*).

Quantitative determinations of heterocyclic amines in cooked foods are reported by Felton et al. (*48*), Hargraves and Pariza (*49*), Hayatsu et al. (*50*), and Turesky et al. (*51*). Recently Grivas and Nyhammar (*52*) and Takahashi et al. (*53*) developed a new method for quantitative determination of heterocyclic amines by HPLC with electrochemical detection. This method increased the sensitivity and, with appropriate prepurification steps, should provide us with a more precise value for amounts of heterocyclic amines in various materials. The recoveries of heterocyclic amines during the procedure were determined by using ^{14}C-labeled compounds (*53*).

Recently Nagao and her associates (*54*) observed the activation of Ha-*ras* oncogene in a rat hepatoma which was induced by IQ feeding. They also found an unknown oncogene, other than *ras*-family or *erb*B, in rat hepatomas induced by IQ. This oncogene can transform NIH 3T3 cells.

Other Mutagens Formed during Cooking

Recently Ohnishi et al. (*55*) observed the formation of mono- and dinitropyrenes in grilled chicken. They calculated that the oral intake of nitropyrenes is much higher than that by inhalation of air polluted with diesel engine exhaust. Nitropyrenes are supermutagens and the carcinogenicity of dinitropyrenes was well documented by Ohgaki et al. (*56,57*), Tokiwa et al. (*58*), and Takayama et al. (*59*).

Nagao and her associates (*60*) found the activated Ki-*ras* gene in a rat fibrosarcoma, induced by repeated subcutaneous injections of 1,8-dinitropyrene.

A wide variety of mutagens are produced as shown by early observations made by Kuratsune (*4*) and Lijinsky and Shubik (*5*). As an example, it could be mentioned that roasting of coffee beans causes mutagenic activity (*61,62*). Some portion of the mutagenicity can be accounted for by methylglyoxal (*63,64*). The production of hydrogen peroxide in brewed coffee and instant coffee solutions was observed under aerobic conditions (*65*). The co-presence of hydrogen peroxide and methylglyoxal showed enhanced mutagenicity.

Table 5. Location of tumors in mice and rats induced by Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, AαC, and MeAαC.

	Mouse	Rat
Trp-P-1	Liver	Liver
Trp-P-2	Liver	Liver
Glu-P-1	Liver	Liver
	Blood vessel	Small and large intestines
		Zymbal gland
		Clitoral gland
Glu-P-2	Liver	Liver
	Blood vessel	Small and large intestines
		Zymbal gland
		Clitoral gland
AαC	Liver	Liver
	Blood vessel	
MeAαC	Liver	Not done
	Blood vessel	

Mutagen Precursors Found in Fermented Foods and Vegetables

Piacek-Llanes and Tannebaum (*66*) first mentioned the presence of a nitrosatable mutagen precursor in fava beans. They postulated a possible relationship between the high incidence of gastric cancers and the intake of fava beans and nitrite in Central and South American countries and searched for mutagens produced by nitrite treatment. The mutagen precursor was later identified as 4-chloro-6-methoxyindole by Yang et al. (*67*). Soybeans were treated by nitrite but did not exhibit any mutagenic activity, while fermented products of soybeans showed mutagenic activity after nitrite treatment. Fermentation is not "cooking" in a strict sense but is linked in various ways with preparation of food.

Two isomers of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids which can be produced by condensation of L-tryptophan and acetaldehyde were found to be mutagen precursors in soy sauce by Wakabayashi et al. (*68*). Tyramine is also found in soy sauce and a mutagenic diazo-oxide compound was produced after nitrite treatment (*69*). A similar diazonium compound was reported to be formed from agalitine in mushrooms and reported to produce tumors in the glandular stomach of mice by Toth et al. (*70*).

From Chinese cabbages, a mutagen precursor was isolated and identified as indole-3-acetonitrile (*71,72*). Pickling procedures may convert nitrate to nitrite and yield acidic conditions, under which the conversion of a mutagen precursor to a mutagen may occur.

Measures To Be Taken for Cancer Prevention with Regard to Mutagen/Carcinogen Formation during Cooking

Mutagens formed during cooking have been newly discovered by adopting the microbial assay. Their struc-

tures were established and they have been synthesized in quantity by organic chemistry. Long-term carcinogenesis experiments gave positive results for compounds tested so far. An important question arises, namely; how significant are these mutagens in human cancer development?

We need to consider that the carcinogenic process is not a simple mutagenic event. In the case of human carcinogenesis, cancers develop over a long latent period after exposure to mutagenic or carcinogenic factors. An obvious example is that atomic bomb survivors developed leukemia, thyroid cancers, and other cancers many years later. Some subjects who accidentally inhaled mustard gas developed bronchial cancer over 10 years later. In experimental systems also, the process of carcinogenesis is composed of multiple steps. Initiation, which may involve a mutational event, and promotion, which follows the initiation step, lead to the completion of carcinogenesis as proven on the mouse skin by Berenblum (*73*). The promotion step on mouse skin is catalyzed by tumor promoters including tetradecanoyl phorbol acetate (TPA) (*74*), teleocidin (*75*), and aplysiatoxin (*76*). The multiple steps of promotion and further progression are proposed for the completion of the carcinogenic processes (*77*). The effects of initiation on various target organs could be readily multiplied and intensified by various factors. Therefore the data on the carcinogenic potential obtained from long-term experiments with rodents given a diet containing a single mutagen can hardly be related directly to human cases. In addition, humans might be different from rodents in their metabolism, repair of DNA modification induced by mutagens and overall susceptibility to carcinogens. Because the completion of the carcinogenic process is the appearance of a tumor arising monoclonally from a single cell, the size of target organs, namely the number of cells existing in target organs should be considered as an important element for more precise risk estimation.

Taking various factors into consideration, it is probably impractical and not realistic to make risk estimations from the carcinogenicity data on rodents given a single carcinogen. However, for a simple extrapolation of animal data for risk estimation, TD_{50} values, which are the doses needed to develop cancers in 50% of animals fed on carcinogens for their life time, have been calculated based on mouse experiments. Those for IQ, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, AαC, and MeAαC were 14.7, 8.8, 2.7, 2.7, 4.9, 15.8, and 5.8 mg/kg/day, respectively. If we assume that the average TD_{50} value of heterocyclic amines should be around 8 mg/kg/day, we can roughly estimate the risk of these carcinogenic heterocyclic amines for human beings. The intake of heterocyclic amines was calculated from available data on their quantities in foods. Apparently the human intake is about 0.0002 times the TD_{50} obtained from animal data. This means that heterocyclic amines may not be so serious for human cancer development. On the other hand, it is also true that humans are being exposed to many heterocyclic amines and many other carcinogens with tumor promoters and/or suppressing factors for carcinogenesis. At this moment, it is honest to state that no solid information on the estimation of risk of heterocyclic amines has been obtained in any direction, either positive or negative. Recently Takayama et al. (unpublished data) found that rats given, simultaneously, five heterocyclic amines—IQ, Trp-P-1, Trp-P-2, Glu-P-2, and AαC—each at a dose of one-fifth those given in independent animal experiments resulted in the production of more multiple colon cancers in a shorter period. It suggests to us that simultaneous exposure to different kinds of carcinogens may yield different carcinogenic responses from those of administrations of single carcinogens. We need further experiments using simultaneous administration of different kinds of carcinogens and those using both carcinogen and tumor promoter treatments.

It should be mentioned that the mutation assay systems commonly adopted are able to pick up carcinogens with relatively high mutagenic activity but miss those with low or no mutagenicity. Mutagenicity cannot be a criterion for the detection of tumor promoters which are probably present in food, and for which other methods should be adopted.

Finally, it would be appropriate to establish a way to avoid the formation of mutagens and carcinogens during cooking by improving heating conditions or by including substance(s) suppressing mutagen formation during cooking.

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