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Influence of amino acids on the formation of mutagenic/carcinogenic heterocyclic amines in a model system

Maria A.E.Johansson[3], Laurent B.Fay[1], Gian A.Gross[1], Kjell Olsson[2] and Margaretha Jägerstad

Department of Applied Nutrition and Food Chemistry, Chemical Centre, Lund University, PO Box 124, S-221 00 Lund, Sweden, [1]Nestec Ltd, Nestlé Research Centre, PO Box 44, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland and [2]Department of Chemistry, Swedish University of Agricultural Sciences, PO Box 7015, S-750 07 Uppsala, Sweden

[3]To whom correspondence should be addressed

Mixtures of creatinine, glucose and various single amino acids were heated at 180°C for 10 min in an aqueous model system. The heated mixtures all showed mutagenic activity, ranging from 80 to 2400 TA98 revertant colonies/μmol creatinine with metabolic activation. Testing of HPLC fractions for mutagenic activity showed each mixture to contain several mutagenic components, some of which corresponded to known heterocyclic amines and others to unknown compounds. The presence of 2-amino-3-methyl-imidazo[4,5-*f*]quinoxaline, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline and 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline in most of the samples was established using HPLC with photodiode array detection and liquid chromatography/mass spectrometry with electrospray interface and single ion monitoring. In addition, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole and the co-mutagenic compounds 9*H*-pyrido[3,4-*b*]indole and 1-methyl-9*H*-pyrido[3,4-*b*]indole were detected in some samples.

Introduction

The major food mutagens isolated to date from cooked meat and fish products are heterocyclic amines (HAs*). Since first reported by Sugimura *et al.* (1), much effort has been devoted to identifying and quantifying these compounds and to understanding the mechanisms of their formation (for reviews see 2,3). Most of the mutagenic heterocyclic amines formed at normal cooking temperatures are imidazoquinolines or imidazoquinoxalines (IQ compounds), imidazopyridines (e.g. 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; PhIP) or imidazofuropyridines (4–6).

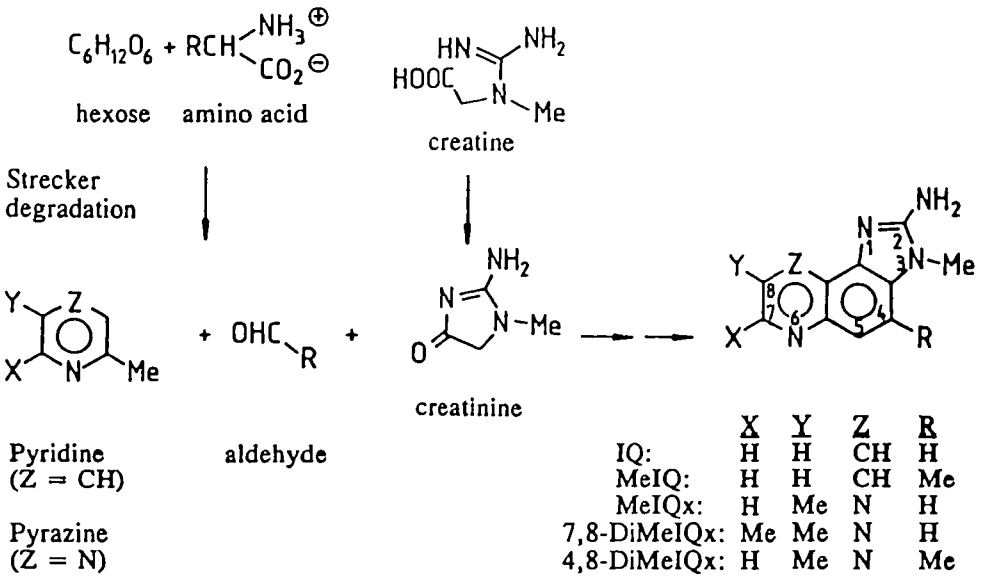
Many HAs are multipotent carcinogens in long-term rodent bioassays (for a review see 7), with 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) also positive in primates (8). Epidemiological studies have shown a relationship between the consumption of fried meat products and an elevated risk of colon and other cancers (9–13). The HAs are animal carcinogens and the IARC classified several of them as possible or probable (IQ) human carcinogens (14–16). Efforts to minimize their formation in cooked foods are therefore of great importance and a better understanding of the formation mechanism is needed. Simple modelling experiments have been useful in providing basic information on precursors, inhibitors and the effect of reaction conditions and a good correlation between the formation of HAs in cooked foods and model systems has been observed (for reviews see 3,17).

All details of the formation mechanism of HAs have not yet been clarified. Jägerstad *et al.* (18) suggested that IQ compounds may be produced via the Maillard reaction from creatin(in)e, amino acids and hexoses present in foods of animal origin (see Scheme 1). This hypothesis has been confirmed by heating creatin(in)e, amino acids and sugars in various model systems, resulting in the formation of IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQx), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx) and PhIP (for reviews see 3,19). Also, ^{14}C-labelled glucose was shown to be incorporated into IQx, MeIQx and 4,8-DiMeIQx using an aqueous model system (20). Alternative routes for the formation of HAs may exist, since their formation from creatin(in)e and amino acids has also been observed in the absence of sugar in dry heating model experiments. However, the yield of HAs in the absence of sugar is usually lower (for a review see 3).

According to the hypothesis illustrated in Scheme 1, amino acids act as precursors of HAs partly by serving as a nitrogen source in pyridine or pyrazine formation (18). However, mutagenic activity and HA yield previously reported in model systems vary depending on the particular amino acid used (for reviews see 3,17). These variations were not completely explained and, so far, the ability to produce mutagenic activity in a model system has been investigated for less than half the naturally occurring amino acids, and even fewer amino acids have been tested as precursors of HAs.

This study was performed to further investigate the effect of various amino acids on the yield and nature of HAs formed in a model system and to increase the understanding of the reactions behind the formation of HAs. Model mixtures, each containing creatinine, glucose and one of the most common amino acids, were heated at 180°C for 10 min. The heated mixtures were tested for mutagenic activity using *Salmonella typhimurium* TA98 with metabolic activation. Furthermore, the model mixtures were purified using the propylsulphonic acid–silica gel tandem extraction method (21) and analysed for known HAs using HPLC with a photodiode array and fluorescence detection and liquid chromatography/mass spectrometry (LC/MS) with electrospray ionization and single ion monitoring. The HPLC fractions were tested for mutagenic activity.

*Abbreviations: HA(s), heterocyclic amine(s); PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; IQx, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline; 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; LC/MS, liquid chromatography/mass spectrometry; 7,8-DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline; 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; 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; harman, 1-methyl-9*H*-pyrido[3,4-*b*]indole; norharman, 9*H*-pyrido[3,4-*b*]indole.



Scheme 1. Suggested pathway for the formation of imidazoquinolines and imidazoquinoxalines (18).

Materials and methods

Chemicals

All chemicals and solvents were of HPLC or analytical grade. The solvents, e.g. acetonitrile, methanol and dichloromethane, were purchased from Merck AG (Darmstadt, Germany). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). Creatinine and amino acids were obtained from Sigma Chemical Co. (St Louis, MO) and glucose from BDH Chemicals Ltd (Dorset, UK). Synthetic IQ, IQx, MeIQ, MeIQx, 4,8-DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), PhIP, 2-amino-6-methyl-dipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) and 1-methyl-9*H*-pyrido[3,4-*b*]indole (harman) were obtained from Toronto Research Chemicals (Downsview, Ontario, Canada) and 9*H*-pyrido[3,4-*b*]indole (norharman) from Aldrich (Steinheim, Germany). The materials used for propylsulphonic acid–silica gel tandem extraction (Extrelut and BondElut, e.g. PRS and C_{18}) were obtained from Merck AG (Darmstadt, Germany) and Analytichem International (Sorbent, Västra Frölunda, Sweden).

Sample preparation

Samples were prepared by heating creatinine, glucose and various amino acids in an aqueous model system as previously described (22). In brief, creatinine (0.9 mmol), glucose (0.45 mmol) and either (0.9 mmol) glycine, alanine, valine, leucine, isoleucine, serine, threonine, aspartic acid, asparagine, glutamic acid, glutamine, lysine, arginine, histidine, phenylalanine, tyrosine, tryptophan, cysteine, cystine, methionine, proline or hydroxyproline were dissolved in 2.5 ml water and heated for 10 min at 180°C in sealed test tubes. A blank sample (without any amino acid) was prepared concurrently.

After heating the samples were purified using the solid phase extraction method of Gross (21) with some minor modifications (22). Using this method a polar extract (containing the IQ-type HAs and glutamic acid pyrolysates) and a non-polar extract (containing pyridoindoles) were obtained. Only the polar extract was analysed for HAs in this study.

HPLC fractionation for mutagenic activity profiles

Extract residues obtained after purification were dissolved in 250 µl HPLC buffer A (see below) and aliquots (30 µl) were injected (Varian 9100 Autosampler) into a Varian 9010 Liquid Chromatograph with a photodiode array UV detector (Varian 9065, Polychrom) equipped with a ToyoSoda TSK Gel ODS 80TM column (250×4.6 mm i.d., 5 µm particle size; Varian, Stockholm, Sweden) and a pre-column (Supelguard LC-18-DB, 20×4.6 mm i.d.) and eluted with a mobile phase of 10 mM aqueous triethylamine adjusted with acetic acid to pH 3.2 (A) or pH 3.6 (B) and acetonitrile (C). A gradient of 5–15% C in A for 10 min, then 15–25% C in B for 10 min and finally 25–55% C in B for 5 min was used. The flow rate was 1 ml/min and the effluent was monitored at 263 nm. Fractions were collected every 30 s between 8 and 30 min and lyophilized before assaying for mutagenic activity.

Table I. Mutagenic activity of model mixtures containing creatinine, glucose and various amino acids heated at 180°C for 10 min

Amino acid	Mutagenic activity[a]
Cysteine	2420
Cystine	2251
Threonine	796
Lysine	556
Serine	483
Alanine	478
Histidine	439
Asparagine	432
Tyrosine	322
Arginine	314
Glutamine	306
Glycine	290
Aspartic acid	271
Methionine	262
Isoleucine	261
Proline	240
Valine	240
Leucine	198
Hydroxyproline	177
Tryptophan	102
Glutamic acid	92
Phenylalanine	83

[a]TA98 revertants/µmol creatinine, with metabolic activation from the linear portion of dose–response curves from replicate platings.

Mutation assay

The mutagenic activity of the lyophilized HPLC fractions was tested as described by Ames *et al.* (23) using *Salmonella* strain TA 98 with the addition of 0.5 ml S9 mix containing 5% chlorophene-induced rat liver/plate (24). The fractions were tested at single doses. Crude heated model mixtures were tested in duplicate at three different doses to establish dose–response curves. The number of revertants/µmol original creatinine was calculated from the linear part of the curve (25). Synthetic MeIQx was used as a positive control (50 000 revertants/µg). The colonies were counted in an automated colony counter using the software Cream™ (Kem-En-Tec, Copenhagen, Denmark). The spontaneous reversion rate was 30–35 revertants/plate. A fraction or sample was considered mutagenic if it induced revertants to twice the background level.

Identification and quantification of HAs using HPLC

The HAs extracted from the heated model systems were identified and quantified by HPLC employing a Hewlett Packard 1090M system containing

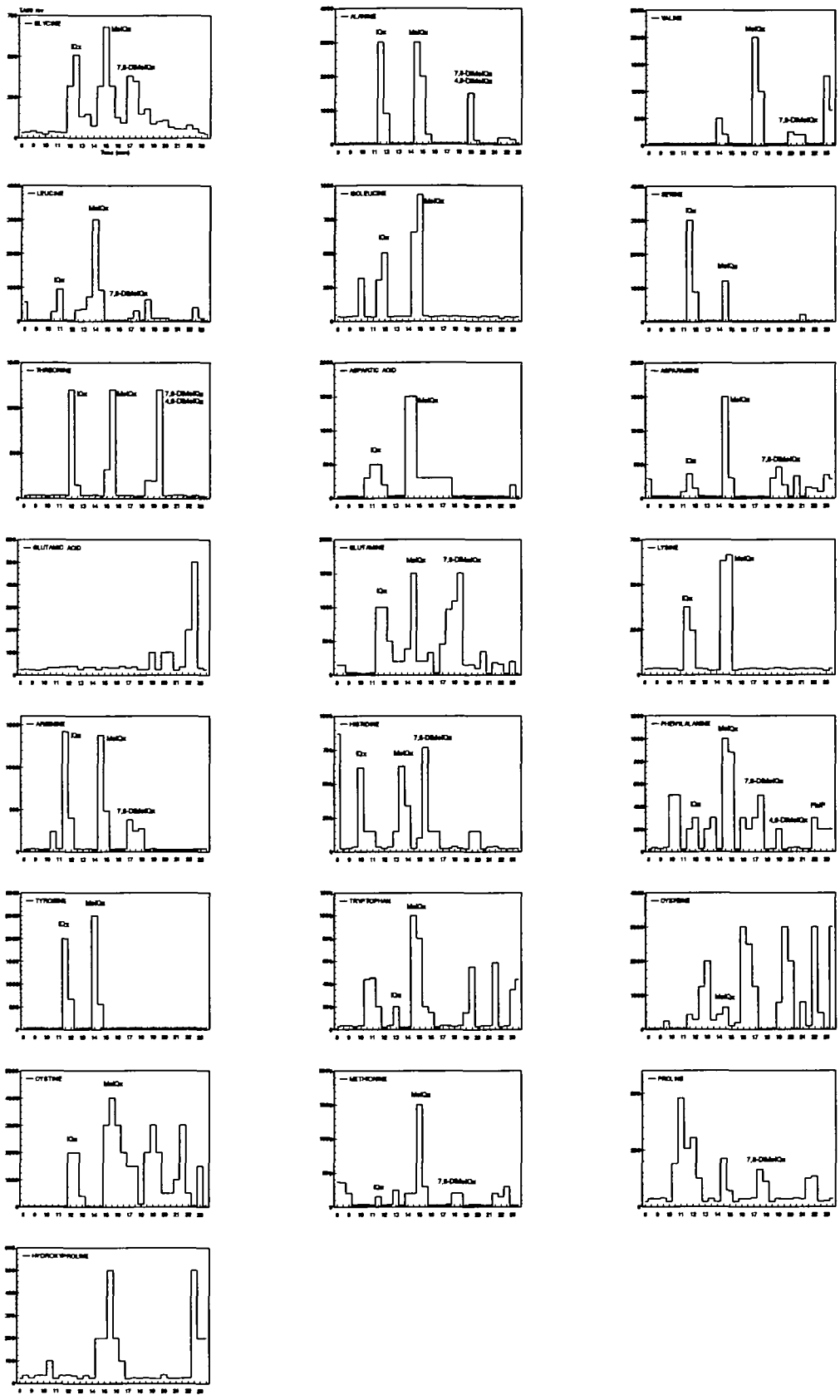


Figure 1. Mutagenic activity in TA98 with metabolic activation in fractions from HPLC separation of heated model mixtures containing various amino acids. Mutagens corresponding to the retention times of HAs are indicated. Retention times of synthetic HAs are: Glu-P-2, 11.3 min; IQ, 11.6 min; IQx, 11.8 min; MeIQ, 14.3 min; MeIQx, 15.1 min; Glu-P-1, 15.3 min; 7,8-DiMeIQx, 17.2 min; 4,8-DiMeIQx, 18.3 min; norharman, 21.1 min; harman, 23.1 min; Trp-P-2, 25.9 min; PhIP, 26.4 min; Trp-P-1, 27.3 min. Retention times of HAs differed due to the complexity of the sample.

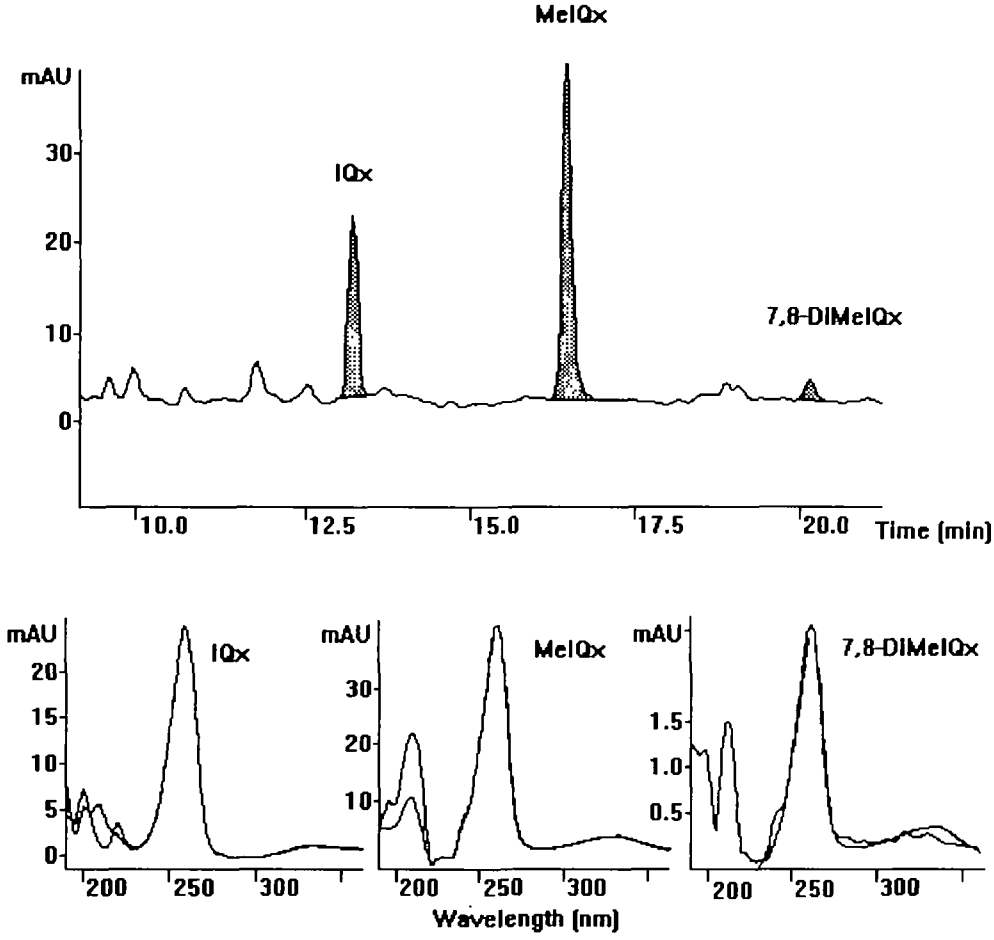


Figure 2. Expanded region of a UV chromatogram (wavelength 263 nm) from HPLC analysis of a heated model mixture containing tyrosine. Peaks corresponding to known HAs are indicated. On-line recorded UV spectra compared with those of synthetic IQx, MeIQx and 7,8-DiMeIQx.

a photodiode array and a time programmable fluorescence detector (HP 1046A) connected in series. Chromatographic conditions were as above. UV detection was performed at 263 nm while excitation/emission wavelengths were 360/450 nm for Glu-P-1 and Glu-P-2, 300/440 nm for harman and norharman, 265/410 nm for Trp-P-1 and Trp-P-2 and 315/390 nm for PhIP. Aliquots of 10 µl from 200 µl of the purified samples were injected.

HAs were identified by comparing the retention times of the peaks with those of synthetic compounds, namely IQ, IQx, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, PhIP, Glu-P-1, Glu-P-2, Trp-P-1, Trp-P-2, norharman and harman, obtained under the same conditions. In addition, some extracts were also spiked with synthetic compounds before injection. UV spectra of synthetic compounds obtained under the same conditions, together with literature data, were used to confirm the identities of the HAs.

The amounts of HAs were estimated by comparing the HPLC peak area of the chromatographed sample with that of a known amount of standard. The amounts were not corrected for incomplete extraction recovery.

LC/MS analysis of HAs

HPLC was performed with a Waters 600-MS pump, a Waters 490-MS UV detector and a Waters 717 autosampler injecting 20 µl of the sample. The column was a ToyoSoda TSK Gel ODS 80TM (250×4.6 mm i.d., 5 µm particle size), protected with a pre-column containing the same stationary phase. The mobile phases were as follows: solvent A, 10 mM ammonium acetate adjusted with HCl to pH 3.2; solvent B, 10 mM ammonium acetate adjusted with HCl to pH 4.0; solvent C, acetonitrile. The linear gradient program was as follows: 0–10 min, 5–17% C in A; 10–10.1 min exchange of buffer B for A; 10.1–22 min, 17–55% C in B; 22–25 min, 55–90% C in B. The flow rate was 1 ml/min and 50% of the flow was split off before entering the mass spectrometer. MS was performed with a Finnigan TSQ-700 mass spectrometer (Bremen, Germany) equipped with a Finnigan electrospray interface working at the high voltage of 4.5 kV. The manifold temperature was 70°C and the heated capillary was set at 250°C. Nitrogen was used as sheath gas at a pressure of 4.8 bar. The compounds were detected after monitoring the protonated molecular ions $[M+H]^+$.

Results

Formation of mutagenic activity in model mixtures

The heated model mixtures all showed mutagenic activity in *Salmonella typhimurium* TA98 with metabolic activation, as shown in Table I. The mutagenic activity ranged from 80 to 2400 revertants/µmol creatinine. Mixtures containing cysteine, cystine or threonine showed the highest response, while the lowest was found in mixtures containing tryptophan, glutamic acid or phenylalanine. No detectable mutagenic activity was formed in the blank sample.

HPLC fractions were tested for mutagenic activity to determine the number of mutagenic compounds and to compare the elution times of the mutagenic components with those of known HAs. Figure 1 shows the mutagenic activity profiles for heated samples containing different amino acids. Each profile contained two to seven mutagenic peaks, some of which elute at similar retention times to those of known HAs found in the pyrolysis products of foods. The mutagenic compounds co-eluting with known HAs are indicated in Figure 1.

Identification of mutagenic compounds

By comparing the retention times of the mutagenic peaks with those of known HAs and by using HPLC UV spectrometry and LC/MS analysis, the presence of several known HAs in the various model systems was established. Figures 2 and 3 show chromatograms obtained from a heated model sample containing tyrosine from HPLC and LC/MS analyses respect-

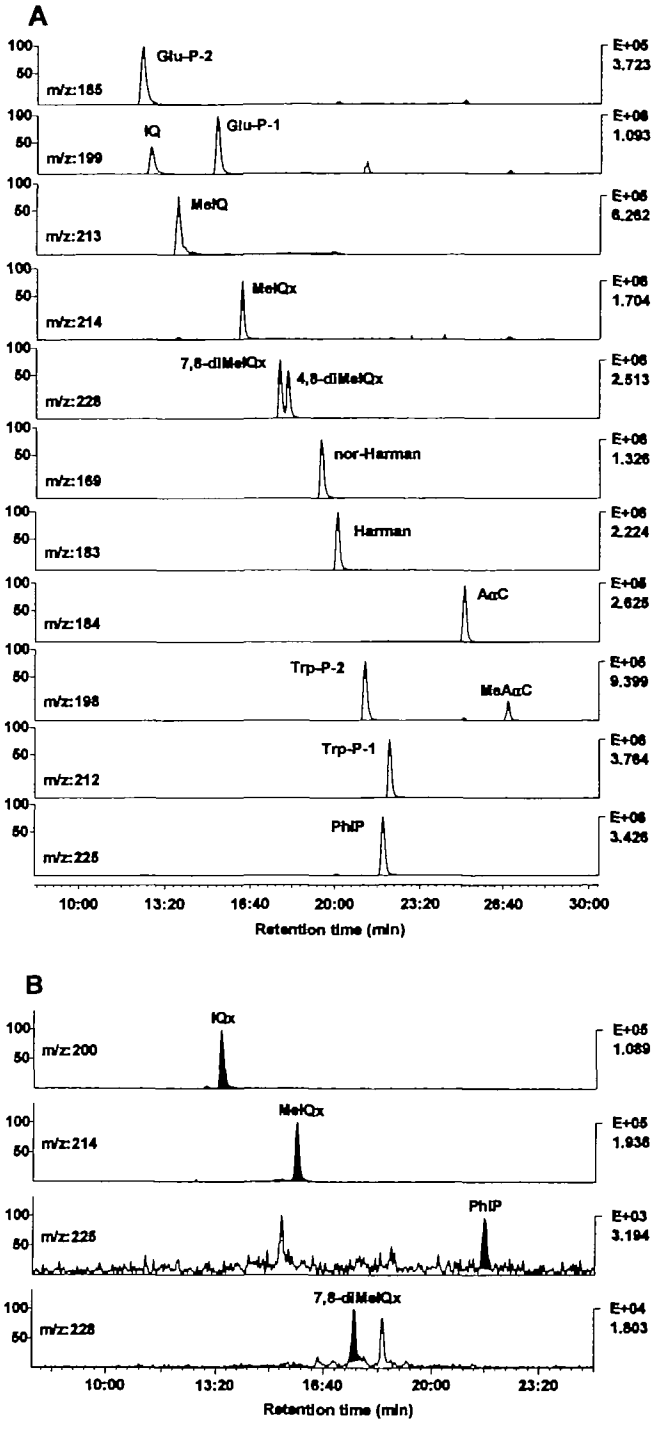


Figure 3. LC/MS analysis of a model system. (**A**) Selected ion monitoring of reference HAs at the respective *m/z* $[M+H]^+$ ions. (**B**) Analysis of a sample containing tyrosine. The chromatogram displays the expanded region containing IQx, MeIQx, PhIP and 7,8-DiMeIQx (shadowed).

ively. A comparision of UV absorption spectra of the tyrosine sample and those of synthetic references is also shown in Figure 2. As shown in Table II, IQx, MeIQx and 7,8-DiMeIQx were present in most of the heated model mixtures. In addition, 4,8-DiMeIQx was detected in samples containing alanine, threonine, lysine, phenylalanine or methionine. We failed to confirm the presence of IQx with LC/MS in some samples, due to unstable signals (Table II). Norharman and harman were formed in samples containing isoleucine, arginine, phenylalanine or tryptophan. Norharman was also formed in a model mixture with tyrosine. PhIP was found in model mixtures containing phenylalanine, isoleucine or tyrosine. Trp-P-1 and Trp-P-2 were found in a model mixture containing tryptophan. Trp-P-1 was also detected in the isoleucine sample. IQ, MeIQ, Glu-P-1 and Glu-P-2 were not found in any of the samples. No known HAs were found in the glutamic acid and hydroxyproline samples or in the blank sample. However, several unknown mutagenic components were present in the heated samples.

Table II. Estimated amounts (μmol/mol creatinine) of IQx, MeIQx and 7,8-DiMeIQx formed in heated mixtures of glucose, creatinine and amino acids analysed using HPLC with photodiode array detection

Amino acid	IQx	MeIQx	7,8-DiMeIQx
Glycine	3.1 ± 0.8	3.6 ± 1.2	0.3
Alanine	5.0[a]	5.4 ± 5.1	0.7
Valine	c	4.7 ± 3.5	c[b]
Leucine	c[a]	2.4	c
Isoleucine	c	2.7 ± 0.7	c
Serine	6.5[a]	3.0 ± 1.7	0.1
Threonine	4.5[a]	9.2 ± 2.1	c
Aspartic acid	c[a]	0.8 ± 0.6	
Asparagine	2.9[a]	1.5	0.2
Glutamine	1.4	0.6	c
Lysine	c[a]	7.7 ± 3.7	c
Arginine	3.7 ± 0.3	4.1 ± 0.7	c[a]
Histidine	1.8	3.4 ± 3.2	c[a]
Phenylalanine	1.5 ± 0.3	1.9 ± 0.7	0.1
Tyrosine	2.6 ± 2.3	3.6 ± 2.1	c
Tryptophan	0.7	5.0 ± 3.5	c
Cysteine		c	
Cystine	0.1	c	
Methionine	0.9[a]	2.7 ± 0.5	c
Proline			c[a]

[a]Not detected using LC/MS.
[b]c, co-eluting compounds interfered with peak area determination.
Amounts are means from the analysis of duplicate samples. LC/MS with electrospray interface and single ion monitoring was used for the detection of the compounds.

Quantification of HAs

The amounts of HAs in the samples were estimated from the UV and fluorescence chromatograms and are shown in Table II for IQx, MeIQx and 7,8-DiMeIQx; their maximum yields were 6.5, 9.2 ± 2.1 and 0.7 μmol/mol creatinine respectively. Samples containing arginine, glycine or tyrosine produced large amounts of IQx. Most MeIQx was formed in the sample containing threonine, followed by samples containing lysine, tryptophan and alanine. The alanine-containing sample produced 1.1 μmol 4,8-DiMeIQx/mol creatinine, while 7.3 ± 4.2 μmol PhIP/mol creatinine were produced in the phenylalanine-containing sample. Due to co-eluting compounds, it was impossible to estimate the amounts of HAs, especially 7,8-DiMeIQx and 4,8-DiMeIQx, in some of the samples. The amounts of norharman, harman, Trp-P-1 and Trp-P-2 were not determined. The amounts of HAs given in Table II are not corrected for incomplete extraction recovery. Extraction of similar model mixtures gave a recovery of 52–97% for MeIQx (19,22).

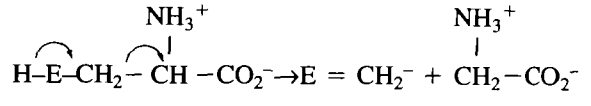
Discussion

The hypothesis for the formation of IQ compounds in Scheme 1 postulated that creatine forms the 2-aminoimidazo part by cyclization and dehydration. The quinoline/quinoxaline part of

the molecule arises from pyridines or pyrazines and Strecker aldehydes, formed in the Maillard reaction (18). Surprisingly, neither IQ nor MeIQ was found in any of the samples. This implies that no pyridines are formed in the model system. IQ and MeIQ have previously been detected in model systems with fructose (26,27) and in dry heating experiments (for a review see 3).

Amino acids act as precursors of HAs by serving as a nitrogen source in pyrazine formation (28). They also seem to provide the carbon and, in some cases, the methyl group at position 4 (18). According to the hypothesis, glycine is a precursor of IQ, MeIQx and 7,8-DiMeIQx, while alanine might give MeIQ and 4,8-DiMeIQx. However, in this study IQx, MeIQx and 7,8-DiMeIQx were formed from most amino acids, including alanine, when heated with glucose and creatinine in the model system. Moreover, 4,8-DiMeIQx was formed not only from alanine, but also from threonine, lysine, phenylalanine and methionine.

Some of these results are hard to reconcile with the hypothesis illustrated in Scheme 1. However, several amino acids are derivatives of alanine carrying an electropositive group (E), e.g. a heteroatom with a free electron pair or an aromatic ring with π electrons. These amino acids, e.g. serine, threonine, cysteine, cystine, phenylalanine, tyrosine, tryptophan and histidine, might undergo retroaldolization to glycine,



thereby explaining the formation of IQx, MeIQx and 7,8-DiMeIQx. The other retroaldolization product (E = CH_2) of threonine is acetaldehyde, which could explain the formation of 4,8-DiMeIQx from threonine. Less reactive retroaldolization products are expected from aromatic amino acids. This might explain why IQx compounds with an aryl group at position 4 have never been observed. It is more difficult to guess which reactions valine, leucine, isoleucine, methionine, asparagine, glutamine, aspartic acid, glutamic acid, proline and hydroxyproline undergo in the model system. One possibility might be that these molecules fragment through free radical reactions. Clearly, the observed IQx compounds do not always fit the hypothesis in Scheme 1 and other reaction pathways cannot be excluded. The divergence might be explained by the preferred formation of other HAs, such as PhIP from phenylalanine and Trp-P-1 and Trp-P-2 from tryptophan.

Pyrazines arise through cyclodimerization of aminodeoxy sugars, formed from α-dicarbonyl compounds and amino acids in the Strecker degradation (29). The yield and species of pyrazines vary with the amino acid (28, 30–33). Sugars are known to fragment through retroaldol or related reactions. If fragmentation precedes pyrazine formation, glyoxal, methylglyoxal and/or biacetyl serve as α-dicarbonyl sources. Methylglyoxal is the most common source, giving rise to 2,5-dimethylpyrazine, which is the precursor of MeIQx and 4,8-DiMeIQx. If glyoxal and methylglyoxal co-dimerize, methylpyrazine, which is the precursor of IQx, is formed. If methylglyoxal and biacetyl co-dimerize, trimethylpyrazine, which is the precursor of 7,8-DiMeIQx, is obtained. Consequently, a mixture of glyoxal, methylglyoxal and biacetyl may have been present in our model mixtures, thus giving rise to IQx, MeIQx, 4,8-DiMeIQx and 7,8-DiMeIQx. However, the methylpyrazines may also have formed through initial cyclodimerization, followed by fragmentation of the pyrazine substituents.

Table III. Literature data on heterocyclic amines in model systems from amino acids and creatin(in)e with and without sugar

Amino acid	Sugar	HA	Yield[1]	Model system[2]	Ref. no.
Gly	Fru	IQ	1.0	reflux	(27)
	Glc	IQx	nd	H_2O	(22)
	Glc	MeIQx	4.4	reflux	(38)
	Fru	MeIQx	6–7	reflux	(27)
	Glc	MeIQx	4	DEG	(35)
	Glc	MeIQx	8.8–17.9	H_2O	(19)
	Glc	MeIQx	7–10	H_2O	(20)
	Glc	4,8-DiMeIQx	nd	DEG	(35)
	Glc	4,8-DiMeIQx	nd	H_2O	(19)
	Glc	7,8-DiMeIQx	1.1	reflux	(39)
	Glc	7,8-DiMeIQx	nd	DEG	(35)
Ala	Fru	MeIQ	nd	reflux	(26)
	Glc	MeIQ	0.9	reflux	(40)
	Rib	MeIQ	1.8	reflux	(40)
	–	MeIQ	nd	dry	(34)
	Fru	4,8-DiMeIQx	1.9–2.6	reflux	(26)
	Glc	4,8-DiMeIQx	4.2	reflux	(40)
	Rib	4,8-DiMeIQx	1.5	reflux	(40)
Leu	–	PhIP	nd	dry	(34)
Ser	–	IQ	3.7	dry	(41)
	–	IQx	2.7	dry	(41)
	–	MeIQx	nd	dry	(34)
Thr	Glc	IQx	nd	H_2O	(20)
	Glc	MeIQx	nd	reflux	(42)
	Glc	MeIQx	9	H_2O	(20)
	Glc	4,8-DiMeIQx	nd	reflux	(42)
	Glc	4,8-DiMeIQx	30	H_2O	(20)
Lys	Rib	MeIQx	4.2	reflux	(40)
	Rib	4,8-DiMeIQx	26.1	reflux	(40)
Tyr	–	MeIQx	nd	dry	(34)
Phe	–	IQ	3.0	dry	(43)
	Glc	IQ	13.5	dry	(43)
	Glc	MeIQx	nd	DEG	(44)
	Glc	4,8-DiMeIQx	nd	DEG	(44)
	Glc	PhIP	3.6	reflux	(45)
	–	PhIP	735	dry	(42)
	Glc	PhIP	560	dry	(42)
	–	PhIP	nd	dry	(34)
	Glc	PhIP	20.9	DEG	(44)
	–	PhIP	6.4	DEG	(44)
Pro	–	IQ	0.4	dry	(46)

[1]Yield in nmol/mmol creatin(in)e.
nd = Not determined.
[2]Reflux = reflux-boiling in diethylene glycol/water (5:1) at 125–128°C for 2 h; H_2O = heated in H_2O in sealed test-tubes at 180°C for 10 min; DEG = heated in deithylene glycol/water (5:1) at 180°C for 10 min; dry = dry heating at 180°C for 1 h.

All amino acids tested produced mutagenic activity to a varying extent when heated with creatinine and glucose in a model system. The mutagenic activity was generally higher than reported earlier. When various amino acids, creatine and glucose were boiled under reflux in aqueous diethylene glycol for 2 h at 128°C only samples containing threonine, glycine or lysine produced mutagenic activity exceeding 200 TA98 revertants/μmol creatine (18). Mixtures containing cysteine and cystine, which produced the highest mutagenic activity in the present study, only showed weak mutagenic activity in our previous study. One explanation might be that a closed model system was used in the present study, preventing volatile intermediates from vaporizing. In another study by Övervik *et al.* (34) various amino acids produced much lower mutagenic activity after dry heating (1 h at 200°C) with creatine in the absence of sugar. In that study samples with serine or threonine

showed the highest mutagenic activity. However, serine and threonine are known to decarboxylate and produce pyrazines through dimerization when heated (29).

Comparing our results with previous studies on the formation of HAs in model systems containing various amino acids (see Table III) shows both similarities and differences. However, different kinds of model systems have been used, e.g. dry heating at 180°C for 2 h, reflux boiling in diethylene glycol/water at 125–128°C for 2 h, heating in diethylene glycol/water at 180°C for 10 min in open test tubes and heating in water at 180°C for 10 min in sealed test tubes.

To our knowledge, this is the first study reporting the formation of IQx and 7,8-DiMeIQx from most amino acids tested (for a review see 3). In addition, several unknown mutagenic components were present in the heated samples. New mutagenic compounds have been first identifed in model systems and later identified in cooked foods (35–37). Much still remains to be done before the complex reaction mechanisms behind the formation of HAs is totally elucidated and the unknown mutagenic compounds are identified.

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