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Short communication

Formation of heterocyclic amine–amino acid adducts by heating in a model system

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ABSTRACT

Although mutagenic and carcinogenic heterocyclic amines (HCAs) are known to be formed in cooked meat and fish, human HCA exposure and carcinogenic risk have not been elucidated in sufficient detail. In this work, we investigated the formations of HCA–amino acid adducts in a model system by using a liquid chromatography–mass spectrometry to elucidate another source of human HCA exposure. The 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) adduct with glycine was formed easily by heating at 200 °C within 5 min, which is probably based on the dehydration condensation of the amino group of PhIP and carboxyl group of glycine. PhIP and other HCAs such as 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinolone, 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline and 3-amino-1,4-dimethyl-5*H*-pyrido[3,4-*b*]indole, also bound with various amino acids by heating. Among these amino acids, proline tends to form adducts with HCAs, but serine, cysteine and lysine hardly bound with HCAs. These results provided a basic understanding of the formation of HCA adducts with amino acids during cooking.

1. Introduction

Mutagenic and carcinogenic heterocyclic amines (HCAs) are formed during heating (broiling, frying, smoking or grilling) of various proteinaceous foods such as meat and fish (Cheng, Chen, & Wang, 2006; Felton et al., 2007; Knize & Felton, 2005; Nagao & Sugimura, 2000; Sugimura, Wakabayashi, Nakagama, & Nagao, 2004; Turesky, 2007). HCAs are activated to DNA-damaging metabolites in the target tissues, and are receiving attention as a risk factor for human cancers, such as breast and prostate cancers (Grover & Martin, 2002; Martin et al., 2010). The formations of HCAs are also studied extensively in different cooked foods and model systems (Alaejos, Ayala, González, & Afonso, 2008; Cheng et al., 2006; Murkovic, 2004; Skog, 2002; Skog, Johansson, & Jagerstad, 1998; Turesky, 2007), and more than 25 HCAs are isolated as mutagens. The amounts of HCAs formed are dependent on the types of meat and fish, method of cooking, and the temperature and duration of cooking (Knize & Felton, 2005; Persson, Sjöholm, & Skog, 2003; Turesky, 2007). 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (PhIP), one of the most abundant HCAs, is found typically in amounts from 0.3 to 182 ng/g, but the levels of other HCAs generally range from undetectable up to 24 ng/g (Nagao & Sugimura, 2000). The estimated daily intake of HCAs in different studies ranges from 58 to 364 ng/day (Nagao & Sugimura, 2000). However, the association between cancer risk and dietary HCA intake has not been clarified in sufficient detail, because the dosages required to induce tumors in rodents are much higher than the estimates of human exposure levels.

On the other hand, genotoxic compounds have been reported to be released by proteolysis of cooked beef (Martin, Cole, Phillips, & Grover, 2002). Recently, we also showed that PhIP is released from high molecular weight compounds by acid hydrolysis or proteolysis of cooked foods, and protein adducts of PhIP are also formed by heating of PhIP and albumin as a model protein (Kataoka et al., 2010). Furthermore, the adduct was estimated to be produced by condensation of the amino group of PhIP and the carboxyl group of the protein from model experiments by heating of PhIP and amino acids. These results suggested that mutagenic HCAs can be released from the HCA–protein adducts by proteolytic digestion in the gastrointestinal tract after a meal, and therefore, these adducts may influence human PhIP exposure and carcinogenic risk.

In complex cooked food matrices, there may be a large number of concurrent reactions that would make the investigation of those reactions in relation to HCA–protein adduct formation difficult. Modelling experiments using standard precursor compounds can simulate simply the adduct formation during cooking. In this study, therefore, we analysed the formation of adducts by heating of several HCAs and amino acids in a model system by liquid chromatography–mass spectrometry (LC–MS) to elucidate the adduct formation during cooking.

2. Materials and methods

2.1. Materials and chemicals

2-Amino-3-methyl-3*H*-imidazo[4,5-*f*]quinolone (IQ) was purchased from Toronto Research Chemicals (Downsview, Canada).

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Table 1
Selected monitoring ions of HCA–amino acid adducts detected in this study.

Amino acid (MW)[a]	SIM ion of HCA–amino acid adduct (m/z)[b]			
	PhIP (224)[a]	IQ (198)[a]	MeIQx (213)[a]	Trp-P-1 (211)[a]
Gly (75)	282	256	271	269
Ala (89)	296	–[c]	–	–
Val (117)	324	–	–	–
Leu (131)	338	–	–	–
Ile (131)	338	–	–	–
Ser (105)	312	286	301	299
Thr (119)	326	–	–	–
Cys (121)	328	302	317	315
Met (149)	356	–	–	–
Pro (115)	322	296	311	309
Asp (133)	340	314	329	327
Glu (147)	354	–	–	–
Asn (132)	339	–	–	–
Gln (146)	353	–	–	–
His (155)	362	336	351	349
Arg (175)	382	–	–	–
Lys (146)	353	327	342	340
Phe (165)	372	346	361	359
Tyr (181)	388	–	–	–
Trp (204)	411	–	–	–

[a] The molecular weights of each precursor HCA and amino acid are shown in parentheses.
[b] Data show m/z of $[M+H]^+$ ion for the HCA–amino acid adduct.
[c] Not determined.

2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), was purchased from Funakoshi Pharmaceutical (Tokyo, Japan). 3-Amino-1,4-dimethyl-5*H*-pyrido[3,4-*b*]indole (Trp-P-1) and PhIP were purchased from Wako Pure Chemical Industries (Osaka, Japan). Each HCA was dissolved in methanol to make a stock solution at a concentration of 1 mg/ml and used after dilution with methanol to the required concentration. Twenty standard amino acids (Gly, Ala, Val, Leu, Ile, Ser, Thr, Cys, Met, Pro, Asp, Glu, Asn, Gln, His, Arg, Lys, Phe, Tyr and Trp) were purchased from Ajinomoto (Tokyo, Japan). Each amino acid was dissolved in 0.01 M HCl to make a stock solution at a concentration of 1 mg/ml and used after dilution with water to the required concentration. LC–MS grade methanol, acetonitrile and distilled water used as mobile phases were purchased from Kanto Chemicals (Tokyo, Japan). All other chemicals were of analytical-reagent grade.

2.2. Formation of HCA–amino acid adducts in a model system

Standard solutions containing HCA (50 μg) and various amino acids (500 μg) were pipetted into 10-ml Pyrex® glass tubes with a PTFE-lined screw-cap, and the mixture was evaporated to dryness in a Model CVE-100 centrifugal evaporator (EYELA, Tokyo, Japan) at 50 °C under reduced pressure. The dried residue was heated on an aluminium dry block heater at 200 °C several times, and the heat-treated product was dissolved in 1.0 ml of MeOH/water (1:3). The solution was analysed by LC–MS after dilution with water to an appropriate concentration and removal of insoluble materials by centrifugation if necessary. In addition, we also analysed samples of HCA or amino acid alone heated in the same manner as controls.

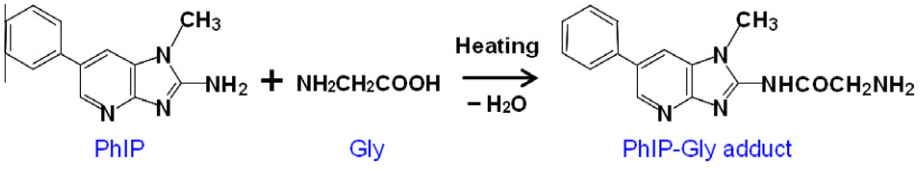

Fig. 1. Formation of PhIP–Gly adduct by heating of PhIP and Gly.

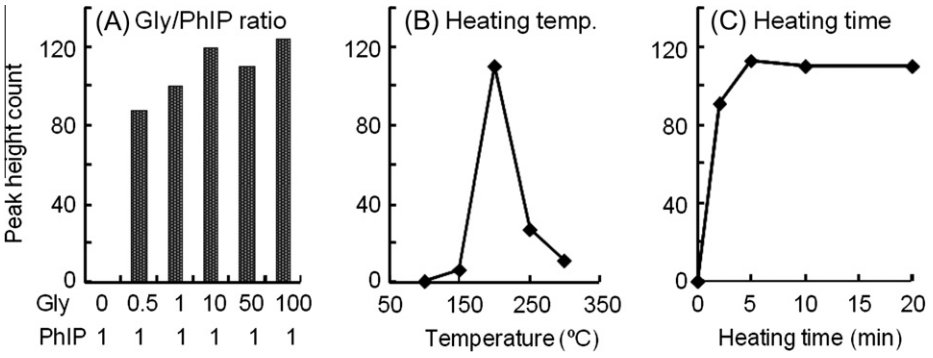

Fig. 2. Effects of heating conditions on PhIP-Gly adduct formation in a model system. (A) Gly/PhIP ratio. (B) Heating temperature for 5 min. (C) Heating time at 200 °C.