RESEARCH ARTICLE

# Formation of protein adducts of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in cooked foods

*Hiroyuki Kataoka[1], Mina Miyake[1], Sachiko Nishioka[2], Tomoko Matsumoto[1], Keita Saito[1] and Kurie Mitani[1]*

[1] School of Pharmacy, Shujitsu University, Okayama, Japan
[2] Faculty of Pharmaceutical Sciences, Okayama University, Okayama, Japan

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Heterocyclic amines (HCAs) are mutagenic and carcinogenic compounds found in cooked meat and fish. Although HCAs are known to form adducts with protein after metabolic activation, adduct formation during cooking has not been elucidated. In this study, we showed that 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is released from high molecular weight compounds by acid or enzymatic hydrolysis of cooked foods. Formation of free and protein adduct forms of PhIP was dependent on cooking temperature and time, and PhIP–protein adducts were estimated to form after formation of free PhIP. We also demonstrated that PhIP–protein adduct is formed by heating of PhIP and albumin as a model protein. A new adduct peak including $[M+H]^+$ ($m/z = 225$) of PhIP as a fragment ion was detected in the high molecular weight fraction of heat-treated protein by LC–MS analysis. From model experiments by heating of PhIP and amino acids, the adduct was estimated to be produced by condensation of the amino group of PhIP and the carboxyl group of protein. PhIP–protein adducts were detected in several cooked meat and fish at ng/g food level as PhIP content. These results suggest that food-borne protein adducts of HCAs may influence human HCA exposure and carcinogenic risk.

**Keywords:**
2-Amino-1-methyl-6-phenylimidazo[4/5-*b*]pyridine / Cooked food / GC–nitrogen-phosphorus detector analysis / LC–MS analysis / Protein adduct

## 1 Introduction

Heterocyclic amines (HCAs) formed during heating (broiling, frying, smoking or grilling) of various proteinaceous foods such as meat and fish are well-known as potent mutagens and carcinogens [1–5]. Exposure to HCAs from the consumption of cooked foods and its effect on human cancer risk are reported [1, 6–8]. To date, more than 25 HCAs have been isolated as mutagens and have been characterized from different cooked foods and several model systems [3, 9–11]. Some HCAs have much higher mutagenic activity than typical mutagens/carcinogens, such as aflatoxin B1, AF-2 and benzo[*a*]pyrene. Among these amines, 3-amino-1,4-dimethyl-5*H*-pyrido[3,4-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido- [3,4-*b*]indole (Trp-P-2), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole, 2-amino-6-methyldipyrido[1,2-α:3′2′-*d*]-imidazole (Glu-P-1), 2-aminodipyrido[1,2-*a*:3′2′-*d*]imidazole (Glu-P-2), 2-amino-3-methyl-imidazo[4,5-*f*]-quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) have been shown to be carcinogenic in rats [12–19] and mice [20–27], and IQ was also shown to be carcinogenic in the monkey [28]. Some of these agents have been classified by the International

**Correspondence:** Dr. Hiroyuki Kataoka, School of Pharmacy, Shujitsu University, 1-6-1, Nishigawara, Okayama 703-8516, Japan
**E-mail:** hkataoka@shujitsu.ac.jp
**Fax:** +81-86-271-7342

**Abbreviations: AαC,** 2-amino-9*H*-pyrido[2,3-*b*]indole; **Glu-P-1,** 2-amino-6- methyldipyrido[1,2-α:3′2′-*d*]imidazole; **HCA,** heterocyclic amine; **IS,** internal standard; **PhIP,** 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; **IQ,** 2-amino-3-methyl-imidazo[4,5-*f*]-quinoline; **MeIQ,** 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; **MeIQx,** 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; **NPD,** nitrogen-phosphorus detector; **Trp-P-1,** 3-amino-1,4-dimethyl-5*H*-pyrido[3,4-*b*]indole; **Trp-P-2,** 3-amino-1-methyl-5*H*-pyrido[3,4-*b*]indole

Agency for Research on Cancer as probable/possible human carcinogens. Furthermore, several case-control studies have reported positive associations between higher consumption of well-done red meat and risks of colon cancer, breast cancer, lung cancer and gastric cancer [29].

HCA contents in cooked foods vary over a range of more than 100-fold and also vary between countries [1, 11, 30–35]. The amounts of HCAs formed are dependent on the types of meat and fish, method of cooking, and the temperature and duration of cooking [2, 5, 36]. 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 [1]. The estimated daily intake of HCAs in different studies ranges from 58 to 364 ng/day [1]. 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 estimates of human exposure levels. Doses of HCAs (IQ, MeIQ, MeIQx, PhIP, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, AαC and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole) that produce tumors in 50% of animals under standard experimental conditions are 0.1–6.4 mg/kg/day in rats and 5.8–64.6 mg/kg/day in mice [37]. However, the carcinogenic potency of HCAs is markedly enhanced in the presence of tumor promoters and agents that cause cell proliferation, and could easily be modulated by other dietary factors, making risk estimation difficult [1]. On the other hand, genotoxic compounds have been reported to be released by proteolysis of cooked beef [38], and they were suggested to be HCAs. Protein adducts are not mutagenic and remain covalently bound, but are considered to release mutagenic HCAs on proteolytic digestion in the gastrointestinal tract after a meal. Therefore, protein adducts of HCAs can become new inducers of mutagenicity in organisms. However, the formation of protein adducts during cooking has not been elucidated in detail, although it is known that HCAs form adducts with proteins (albumin or hemoglobin) after metabolic activation in the human body [1, 39–42]. Such protein adducts would not be extractible by any of the commonly used procedures for HCA isolation and may not have been included in determined levels, and therefore may have been omitted from total daily intake estimations. In this study, we demonstrated that protein adducts of PhIP form by cooking and PhIP is released by acid or enzymatic hydrolysis of heat-treated protein. Moreover, we measured the presence and contents of protein adducts of PhIP in various fishes and meats prepared by common cooking procedures.

## 2 Materials and methods

### 2.1 Materials and chemicals

In this study, the following ten HCAs were used for the analysis. IQ was purchased from Toronto Research Chemicals (Downsview, Canada). MeIQ, MeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo[4,5-*f*]- quinoxaline (7,8-DiMeIQx) and AαC were purchased from Funakoshi Pharmaceutical (Tokyo, Japan). Trp-P-1 and PhIP were purchased from Wako Pure Chemical Industries (Osaka, Japan). Trp-P-2 and Glu-P-1 were kindly provided by Dr. H. Hayatsu, Professor Emeritus of Okayama University. 2-Amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline (4,7,8-TriMeIQx; Funakoshi) was used as an internal standard (IS). 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 require concentration. DMF dimethylacetal was purchased from Nacalai Tesque (Kyoto, Japan). Twenty amino acids, glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), serine (Ser), threonine (Thr), cystein (Cys), methionine (Met), proline (Pro), aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), glutamine (Gln), histidine (His), arginine (Arg), lysine (Lys), phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp), were purchased from Ajinomoto (Tokyo, Japan) and used for PhIP–adduct formation in a model system. Bovine serum albumin, human blood hemoglobin and bovine milk casein were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Blue-rayon® was obtained from Funakoshi. LC–MS grade methanol, ACN and distilled water used as mobile phases were purchased from Kanto Chemicals (Tokyo, Japan). All other chemicals were of analytical-reagent grade.

### 2.2 Preparation and fractionation of food samples

Food samples were purchased at local retail markets and were treated on the same day. Fish and meat samples (100–200 g) were weighed and cooked at 200°C for 5 min per side. Four types of fresh fishes including pacific saury, cod, mackerel and salmon were grilled. Pan-fried beef, stir-fried pork and grilled chicken were used as meat sample. In addition, pan-fried chicken egg, dried-cuttlefish and bread were also included as samples. These samples were cooked at 200°C for 3 min. Outline of sample preparation and fractionation of above cooked food samples is shown in Fig. 1. Homogenates of each cooked sample were separated by extraction with dichloromethane. The dichloromethane extractable fraction was used for the analysis of HCAs as the ''free fraction''. On the other hand, the precipitate (non-extractable fraction) was used for the analysis of protein adducts as the ''protein fraction''. Acid hydrolysis of the protein fraction was carried out as follows. Aliquots (20–50 mg) of the powdered protein fraction were hydrolyzed with 0.2 mL of 6 M HCl in the vapor phase at 100°C for 3 h under vacuum in a Pico-Tag workstation (Waters Assoc., Milford, MA, USA). Enzymatic digestion of the protein fraction with proteinase K was carried out at 37°C for 48 h by modification of the method reported

 

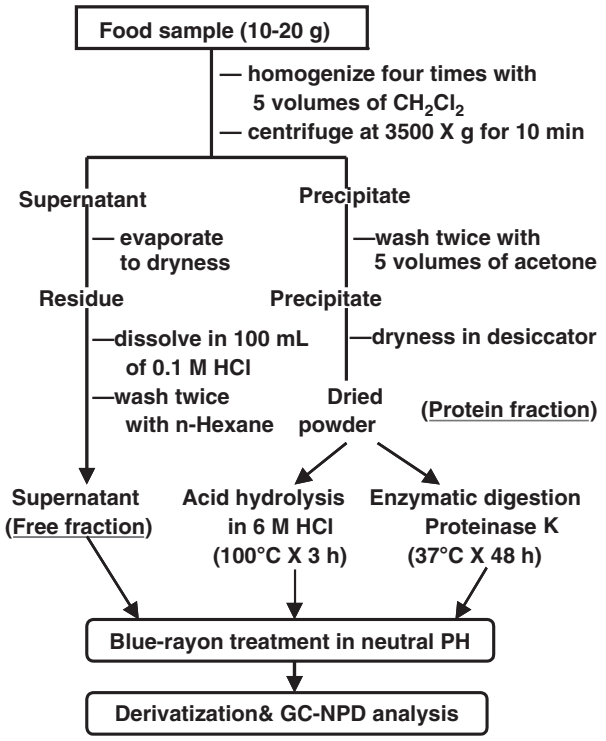


**Figure 1.** Outline of sample preparation and fractionation of cooked food samples.

previously [38]. The free fraction and the acid or enzymatic hydrolysate of the protein fraction were neutralized with 6 M NaOH, and insoluble materials were removed by filtration if necessary. HCAs in the solution were extracted with Blue-rayon® (150 mg) by modification of the method reported previously [30]. The HCAs adsorbed on the Blue-rayon® were eluted three times with MeOH–28% $NH_3$ (50:1) and the combined eluate were evaporated to dryness at 50°C under reduced pressure. The residue was washed with *n*-hexane under acid condition (pH < 2) and extracted with dichloromethane under $NH_3$ alkaline condition (pH > 10), and then the extract was used for derivatization of the sample.

### 2.3 Derivatization and GC–NPD analysis of HCAs

Derivatization of HCAs was performed by the method reported previously [30]. Briefly, an aliquot of the sample containing 0.5–10 ng of HCAs was pipetted into a Pyrex glass tube with a PTFE-lined screw-cap. To this solution was added 10 ng of IS (if necessary) and 10 μL of DMF dimethylacetal, and the mixture (0.2–0.5 mL) was heated at 100°C for 15 min without capping the tube. After evaporation to dryness, the residue was dissolved in 20–40 μL of ethyl acetate and then 1 μL of this solution was injected into the GC. GC conditions were as follows: separation column, two connected fused-silica capillary columns (J&W Scientific, Folsom, CA, USA) containing DB-17 ht (10 m × 0.25 mm id, film thickness 0.15 μm) and DB-1 (10 m × 0.25 mm id, film thickness 0.25 μm); column temperature, programmed at 10°C/min from 240 to 290°C, programmed at 20°C/min from 290 to 330°C and held at 330°C for 2 min; injection and detector temperature, 340°C; inlet helium pressure, programmed at 4 kPa/min from 185 to 205 kPa, programmed at 10 kPa/min from 205 to 225 kPa and held at 225 kPa for 1 min; make-up gas flow-rate: 30 mL/min; split ratio: 10:1; detector, nitrogen-phosphorus detector (NPD). The peak height ratios of HCAs and the IS were measured and the peak height ratios relative to the IS were calculated to construct calibration curves.

### 2.4 Formation of PhIP–protein adducts in a model system

Albumin, hemoglobin and α-casein were used as model proteins for demonstration of the adduct formation by heating of PhIP and protein. Each protein (50 mg) was weighed into a 10 mL Pyrex glass tube with a PTFE-lined screw-cap and 0.5 mL of 1.0 mg/mL of PhIP was added, and the mixture was evaporated to dryness at 50°C under reduced pressure. The dried residue was heated on alminum dry block heater at 200°C for 10 min and the heat-treated protein was then washed to remove coexisting PhIP by each twice with 2 mL of dichloromethane and acetone. The residual protein fraction was dissolved in 0.5 mL of water and then 100 μL of this solution was injected into the LC–MS after filtration with syringe filter in necessary. In addition, we also analyzed samples of PhIP or protein alone heated in the same manner as controls.

### 2.5 Formation of PhIP–amino acid adducts in a model system and LC–MS analysis

Standard solutions containing PhIP (50 μg) and various amino acids (500 μg) were heated at 200°C for 5 min as above, and the heat-treated product was dissolved in 1.0 mL of MeOH/water (1:3). The solution was analyzed by LC–MS and LC–MS/MS after dilution with water to an appropriate concentration and removal of insoluble materials by centrifugation if necessary. In addition, we also analyzed samples of PhIP or amino acid alone heated in the same manner as controls.

### 2.6 Instrument and analytical conditions

LC–MS analysis was carried out with a Model 1100 series LC coupled with a diode array detector and an atmospheric pressure ESI (AP-ESI) MS (Agilent Technologies, Boeblingen, Germany). For the analysis of PhIP–protein adducts,

 

an Inertsil WP300 C8 column (100 mm × 4.6 mm, particle size of 5 μm; GL Science, Tokyo, Japan) was used for LC separation under the following conditions: column temperature, 30°C; mobile phase, 5 mM ammonium formate; and flow rate, 0.5 mL/min; run time, 15 min. ESIMS conditions were as follows: nebulizer gas $N_2$ (35 psi); drying gas, $N_2$ (12 L/min, 350°C); fragmenter voltage, 80 V; capillary voltage, 3000 V; ionization mode, positive mode; mass scan range, 100–3000 Da; selected monitoring ion, *m/z* 225 for PhIP. For the analysis of PhIP–amino acid adducts, an LC–CN column (75 mm × 3.0 mm, particle size of 3 μm; Supelco, Bellefonte, PA) was used for LC separation under the following conditions: column temperature, 30°C; mobile phase, 5 mM ammonium formate/ACN (75:25, v/v); and flow rate, 0.2 mL/min; run time, 15 min. ESIMS conditions were as follows: nebulizer gas $N_2$ (40 psi); drying gas, $N_2$ (12 L/min, 350°C); fragmenter voltage, 90 V; capillary voltage, 3000 V; ionization mode, positive mode; mass scan range, 70–450 amu. LC–MS data were processed with an HP ChemStation (Hewlett-Packard, Palo Alto, CA).

## 3 Results and discussion

### 3.1 Analysis of the formation of HCA-protein adducts during cooking of foods

Food samples were cooked using common cooking method, although the formation of HCAs is dependent on cooking time and temperature. Cooked foods were fractionated into the ''free fraction'' containing low molecular weight compounds and the ''protein fraction'' containing high molecular weight compounds by solvent extraction [30, 38]. Low molecular weight HCAs were extracted quantitatively into dichloromethane from cooked foods and separated from coexisting substances by the Blue-rayon® extraction method [43], which can selectively adsorb HCAs. The protein fraction was separated as the water-soluble precipitate by dichloromethane extraction and free HCAs were not detected in this fraction. To confirm the bonding of PhIP in the protein fraction, the release of PhIP by acid hydrolysis was optimized. Maximum amount of PhIP released from adducts was obtained at 100°C for 3 h. Figure 2 shows GC–NPD chromatograms obtained from the protein fraction of cooked fish with and without acid hydrolysis under optimum conditions. As shown in Figs. 2B and C, PhIP was appeared in the chromatogram obtained from the acid hydrolysate of the protein fraction. Other HCAs were not detected in the acid hydrolysate. A neighboring peak detected in protein fraction (Fig. 2B) is different in retention time of MeIQx clearly and MeIQx was not detected by LC–MS analysis of acid hydrolysate.

PhIP contents in free and protein fractions of cooked meat and fish were measured by the GC–NPD method. As shown in Table 1, PhIP contents in the protein fraction were 1/3–1/2 the level of free PhIP contents. PhIP was also released from the protein fraction by proteolysis. The data obtained by enzymatic digestion are not quantitative, because proteolysis conditions were referred to previous report [38] (not optimized). However, these results suggest that PhIP binds covalently with proteins. Several peaks for the PhIP–protein adducts were observed in the protein fraction of cooked meats by Sephadex G-100 gel chromatography: 8 mL of the protein fraction was applied to the column (32 cm × 2 cm id) and water eluate was collected

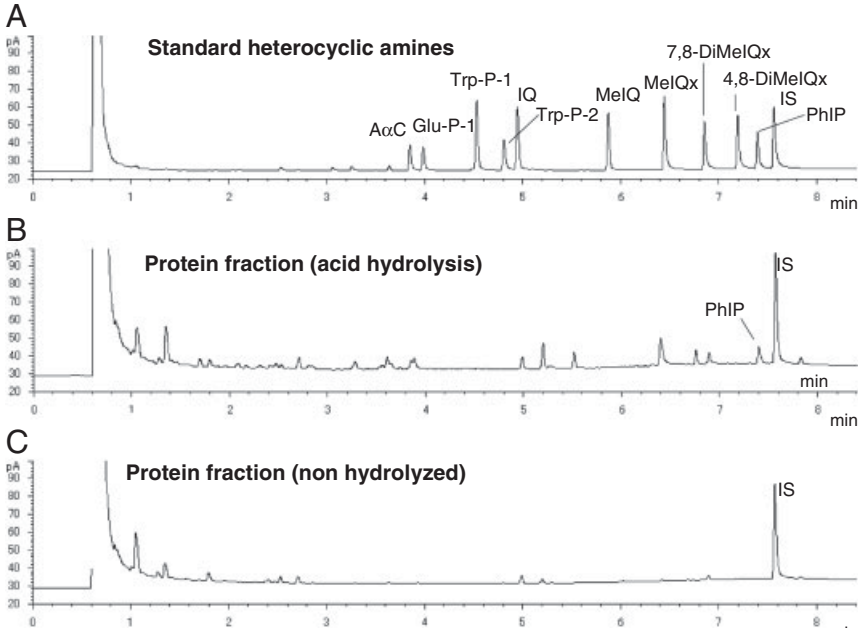


**Figure 2.** GC–NPD chromatograms obtained from standard HCAs and protein fraction of cooked fish. (A) Standard solution (20 ng/mL of each HCA), (B) acid hydrolysate of protein fraction and (C) non-hydrolyzed protein fraction. See Section 2 for GC–NPD conditions.

 

**Table 1.** PhIP contents in free and protein fractions of uncooked and cooked foods

| Food sample | PhIP content[a] (ng/g sample) | | |
|---|---|---|---|
| | Free fraction | Protein fraction | |
| | | Acid hydrolysis | Proteolysis |
| Uncooked beef | ND[b] | ND | ND |
| Cooked beef[c] | 3.79 (3.74, 3.84) | 1.83 (1.79, 1.87) | 0.95 (0.92, 0.98) |
| Cooked mackerel[c] | 8.94 (8.31, 9.57) | 3.33 (3.03, 3.63) | 0.93 (0.63, 1.23) |

a) Each data shows an average of duplicate analyses. Parentheses show the minimum and the maximum.
b) Not detectable.
c) Food samples were cooked at 200°C for 5 min per side.

3 mL/tube for measurement of UV absorbance at 280 nm and PhIP detection by GC–NPD. The molecular weight of one peak in these adducts was estimated with about 10 622 by deconvolution LC–MS analysis, and it is considered to be PhIP adduct with protein fragment.

To evaluate the PhIP–protein adduct formation during cooking, meat was cooked with several heating times (2–10 min) and temperatures (100–300°C). As shown in Fig. 3, the formation of free and protein adduct of PhIP increased with both increasing time and temperature. PhIP content in the protein adduct was initially higher than free PhIP content, but the free PhIP content increased gradually and PhIP content in the protein adduct reached a plateau. These results suggested that the free PhIP initially formed in cooked food is incorporated into PhIP–protein adduct formation. Furthermore, PhIP–protein adduct tends to be decomposed with longer heating time and higher temperatures.

### 3.2 Analysis of formation of PhIP–protein adduct in a model system

To examine the mechanism of the protein adduct formation during cooking, the formation of PhIP–protein adduct was analyzed by heating of PhIP and a model protein. Albumin, hemoglobin and casein were used as model proteins. The mixtures of PhIP and protein were heated at 200°C for 10 min and then analyzed by LC–MS without hydrolysis. As shown in Fig. 4, a new adduct peak including $[M+H]^+$ ($m/z = 225$) of PhIP as a fragment ion was detected in the protein fraction obtained from the heat-treated albumin–PhIP mixture by LC–MS analysis using a WP300 C8 column. This peak was not detected in unheating samples or control samples of heated albumin only. Although standard PhIP is eluted more late since 15 min in this column (not shown in Fig. 4), the PhIP not reacted during heating is removed by solvent extraction prior to the LC–MS analysis. Although a peak being retention times around 6.5–7.0 detected by the DAD was newly observed in the reaction mixture of albumin and PhIP, it was not detected by the MS (scan range at 100–3000 Da). It is considered that this material can not be ionized in positive mode or the mass is out of determination range. On the other hand, PhIP–protein adducts with hemoglobin and casein were not detected. These results suggest that PhIP–protein adducts form after the formation of free PhIP, although the adduct formation ability differs with protein source.

### 3.3 Analysis of formation of PhIP–amino acid adduct in a model system

To confirm HCAGly adduct formation, the mixtures of Gly (1.0 mg) and PhIP (0.1 mg) were heated at 200°C for 10 min in a dry model system. As shown in Figs. 5E and F, a new adduct peak was observed on chromatogram between peaks of Gly and PhIP, and $[M+H]^+$ with postulated $m/z = 282.4$ produced by condensation of these compounds was detected in the mass spectrum of this peak (Fig. 5G). This peak was not detected in control samples of heated Gly (Figs. 5A and B) or PhIP (Figs. 5C and D) only. In addition, prominent fragment ion peaks at $m/z = 236.9$ [M+H45 ($CH_3$ and $CH_2NH_2$)], $m/z = 225.4$ $[PhIP+H]^+$ and $m/z = 73.1$ ($NHCOCH_2NH_2$) were observed by LC–MS/MS analysis of this peak (Fig. 5H). From these results, this adduct was estimated to be occurred by condensation of the amino group of PhIP and the carboxyl group of Gly.

To compare the formation efficiency of the PhIP–amino acid adducts, PhIP (50 μg) and amino acid (500 μg) were heated at 200°C for 5 min as a model system. The $[M+H]^+$ of each adduct generated by condensation was analyzed by LC–MS with SIM mode detection, and the formation efficiency was evaluated by peak height count. As shown in Fig. 6, PhIP bound covalently with all amino acids except for Ser, Thr, Cys, Asp, Asn and Lys. Among the amino acids tested, Pro tends to form adducts with PhIP. These results provided a basic understanding of the formation of PhIP adducts with amino acids and proteins during the cooking of meat and fish.

 

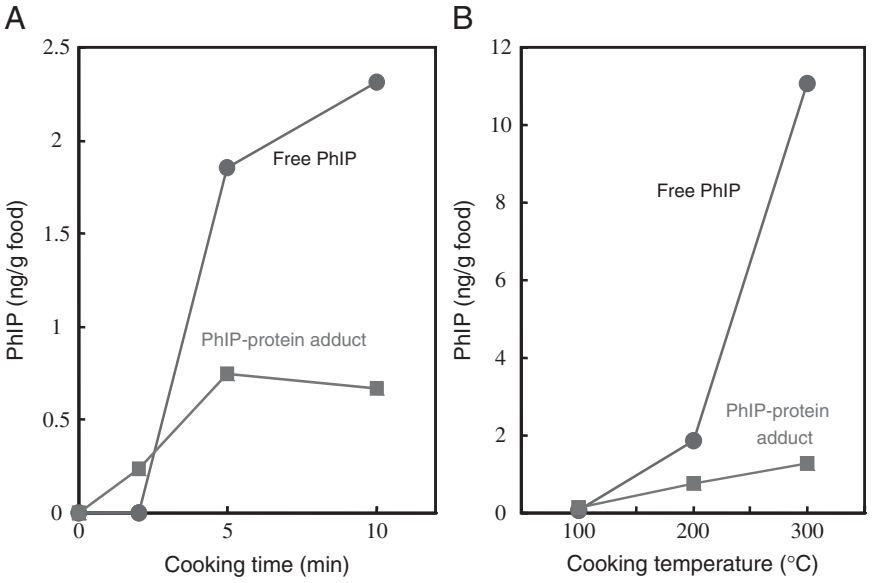


**Figure 3.** Effects of cooking conditions on the formation of free PhIP and PhIP–protein adducts in cooked beef. (A) Cooking time at 200°C and (B) cooking temperature for 5 min.

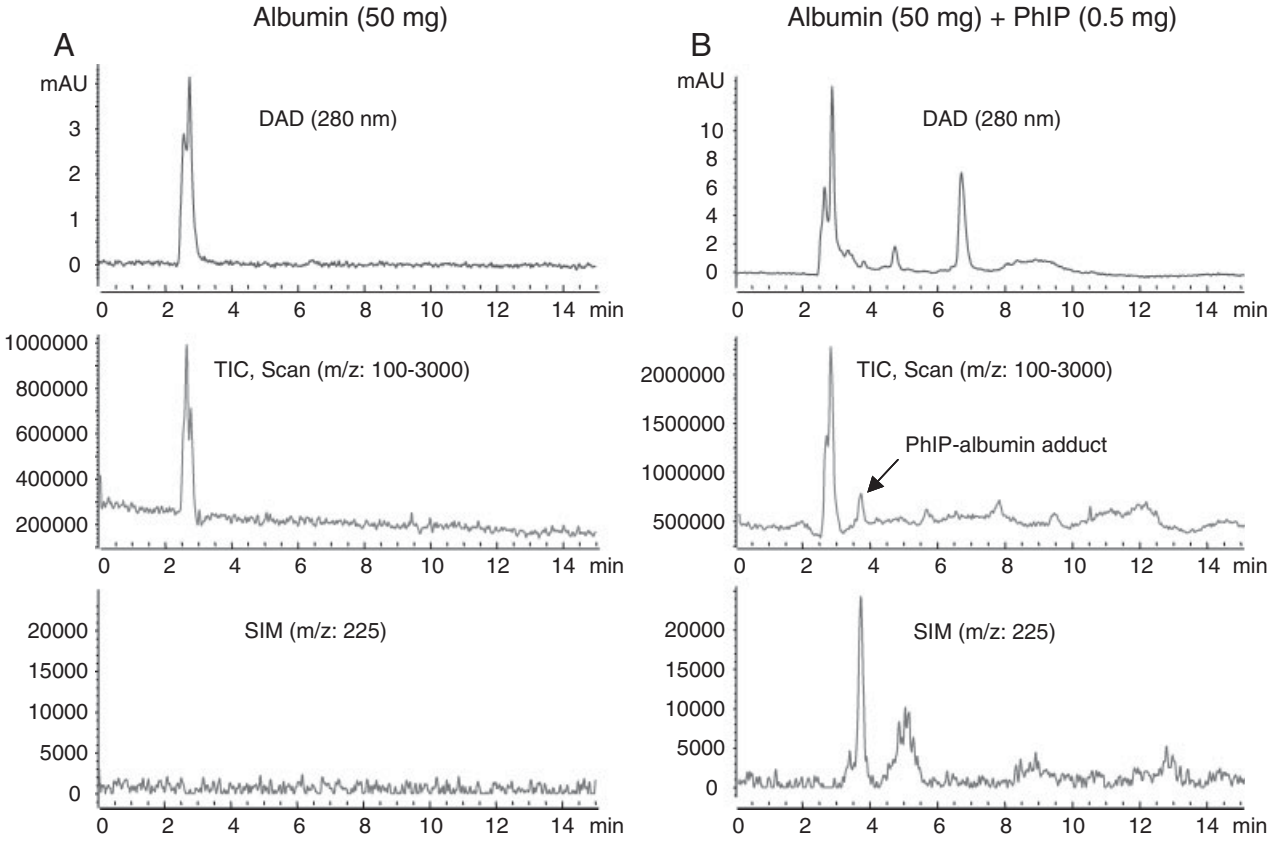


**Figure 4.** LC–MS chromatograms obtained by heating (at 200°C for 10 min) of (A) albumin and (B) albumin+PhIP. See Section 2 for LC–MS conditions.

 

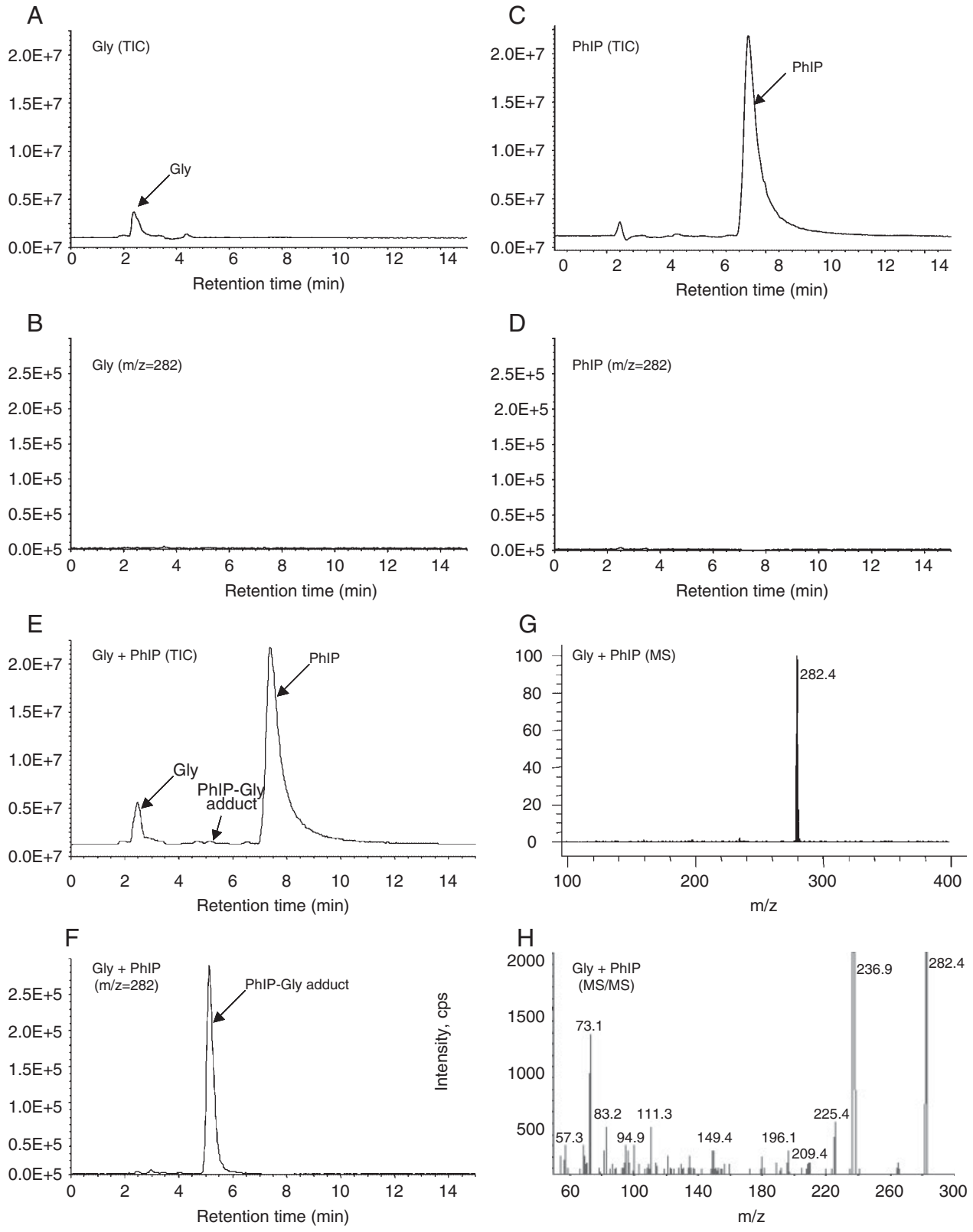


**Figure 5.** Chromatograms and mass spectra of PhIP-Gly adducts formed by heating at 200°C for 10 min. (A), (C) and (E): Total ion chromatograms obtained from heated Gly, PhIP and Gly+PhIP. (B), (D) and (F): Mass chromatograms ($m/z = 282$) obtained from heated Gly, PhIP and Gly+PhIP. (G) Mass spectrum of PhIP-Gly adduct. (H) Tandem mass spectrum of PhIP-Gly adduct ($m/z = 282.4$). See Section 2 for LC–MS conditions.

 

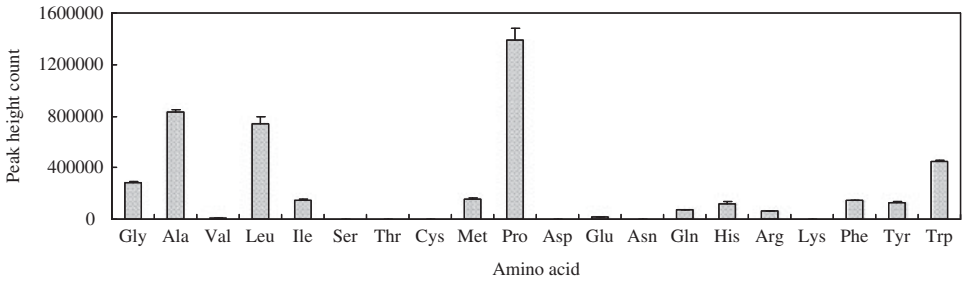


**Figure 6.** Effects of amino acids on the formation of PhIP–amino acid adducts by heating (at 200°C for 5 min) in a model system. Data are shown as averages of three analyses.

**Table 2.** PhIP–protein adduct contents in several cooked foods

| Cooked food | Content[a)] (ng/g food) |
|---|---|
| Saury | 9.00 (8.01, 9.99) |
| Cod | 8.74 (8.56, 8.92) |
| Mackerel (white flesh) | 0.90 (0.84, 0.96) |
| Mackerel (red flesh) | 1.59 (1.42, 1.76) |
| Salmon | 4.92 (4.32, 5.52) |
| Dried cuttlefish | ND[b)] |
| Beef | 0.75 (0.68, 0.82) |
| Pork | 1.37 (1.26, 1.48) |
| Chicken | 0.65 (0.60, 0.70) |
| Egg yolk | 0.61 (0.54, 0.68) |
| Egg white | 0.65 (0.58, 0.72) |
| Bread | ND |

a) Contents of PhIP–protein adduct are shown as PhIP content in protein fraction of cooked food. Each data shows an average of duplicate analyses. Parentheses show the minimum and the maximum.
b) Not detectable.

### 3.4 Contents of PhIP–protein adducts in cooked foods

The contents of PhIP in protein adducts in various foods prepared under common cooking conditions were analyzed by GC–NPD analysis. As shown in Table 2, PhIP–protein adducts were detected in some cooked meat and fish samples at the ng/g food level as PhIP content. These results suggested that food bone protein adducts of PhIP may influence human PhIP exposure and carcinogenic risk. Therefore, the presence of such adducts may be an important factor for evaluation of carcinogenic risk of HCAs, because a portion of the HCAs formed by cooking change into non-mutagenic adducts, and may then be altered into mutagens by biodegradation.

## 4 Concluding remarks

Formation of protein adducts of PhIP in cooked foods was demonstrated. PhIP-protein adduct was formed by heating of PhIP and albumin, and it was confirmed by LC-MS analysis. The adduct was estimated to be produced by condensation of the amino group of PhIP and the carboxyl group of protein from model experiments by heating of PhIP and amino acids. These results suggest that protein adducts of HCAs may influence carcinogenic risk. Further work should be carried out to evaluate dynamics of the adducts in organism after food intake, and their contribution for carcinogenicity.

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*The authors have declared no conflict of interest.*

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