DOI 10.1002/mnfr.200800206 Mol. Nutr. Food Res. 2009, *53*, 716–725

Research Article

Inhibition of mutagenic PhIP formation by epigallocatechin gallate *via* scavenging of phenylacetaldehyde

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Chemical model investigation showed that both epigallocatechin gallate (EGCG) and its peracetate, which has all the hydroxyl groups acetylated, effectively reduced the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the most abundant mutagenic heterocyclic amine found in foods. Mechanistic study was subsequently carried out to characterize the probable inhibitory mechanism involved. GC-MS analysis showed that EGCG in only one-fourth molar quantity of phenylalanine reduced formation of phenylacetaldehyde, a key PhIP intermediate by nearly 90%. Its peracetate also showed similar inhibitory activity. This further supported the existence of an antioxidant-independent mechanism contributing to the inhibition of PhIP formation by EGCG. Subsequent LC-MS analyses of samples from a wide range of model systems consisting of PhIP precursors showed the generation of characteristic analytes with molecular weight corresponding to the sum of EGCG and phenylalanine fragment(s) only in models where phenylalanine and EGCG were simultaneously present. An isotope-labeling study revealed that these analytes all contained fragment(s) of phenylalanine origin. Direct reaction employing phenylacetaldehyde and EGCG further confirmed the capability of EGCG to form adducts with phenylacetaldehyde, thus reducing its availability for PhIP formation. Finally, an investigation of the time course of the generation of postulated adduction products supported EGCG as an effective inhibitor of PhIP formation in prolonged heating processes.

Keywords: Adducts / Chemical model reactions / Epigallocatechin gallate peracetate / Phenylacetaldehyde / PhIP

Received: May 26, 2008; revised: August 7, 2008; accepted: August 18, 2008

1 Introduction

Heterocyclic amines (HAs) belong to a group of potent mutagenic/carcinogenic compounds associated with heat-processed muscle foods. Many HAs have demonstrated carcinogenic activity in rodents [1, 2] and one of them, 2-amino-3-methylimidazo[4,5-*f*]quinoline, induced tumors in non-human primates [3]. 2-Amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) occurs most frequently and in the greatest abundance in foods [4, 5]. Many lines of research in the fields of food chemistry and toxicology have contributed to our understanding of its routes of metabolism in mammals as well as its mutagenic and carcinogenic activity and mechanisms [6–8]. PhIP has been shown to cause tumors in animal models [9], and exposure to PhIP increased the risk of mammary carcinogenesis in the second generation, probably *via* a transplacental route or *via* its secretion into the milk [10]. The liver, mammary gland, colon and prostate have been identified as major target organs of carcinogenesis of HAs in animal studies [8, 11–14]. Recent studies have reported the detection of PhIP-DNA adducts from these tissues in humans [15–17]. In addition, epidemiological evidence has also supported a positive correlation between red meat consumption, PhIP intake and cancer of these tissues [18–21].

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Abbreviations: **EGCG**, epigallocatechin gallate; **HA**, heterocyclic amines; **PhIP**, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; **RCS**, reactive carbonyl species

Reducing dietary exposure to HAs may be the most practical way of minimizing HA-associated health risk. Several strategies have been proposed such as avoiding prolonged heat treatment at high temperature [22, 23] and marinating meat with certain natural ingredients before cooking [24, 25]. In this regard, our group has carried out a series of experiments aiming to develop effective inhibitory strategies that could easily be adopted by the general public. Both natural extracts and pure phytochemicals have been explored, and we found that phenolics such as procyanidins, flavonones and flavan-3-ols significantly decreased the formation of HAs, including PhIP [26, 27]. Our and other studies [28, 29] have identified tea polyphenols, especially catechins, as being among the most effective natural inhibitors of PhIP formation. Until recently, there have been only limited studies on the inhibitory mechanism of phytochemicals on HA formation, especially PhIP. Although previous reports tend to ascribe polyphenols' inhibitory activity on HA formation to their antioxidant capacity, our preliminary mechanistic investigation employing a large group of natural polyphenols suggested that antioxidation might not be the dominant inhibitory mechanism of phenolic compounds against HA formation [26]. The lack of a significant positive correlation between these two activity parameters could also be inferred from findings of another study [29]. This implies that alternative mechanism(s) of action could exist for many of these phenols. Both free radicals and Maillard reaction products including reactive carbonyl species (RCS) arising from thermal and/or Strecker degradation reactions have been proposed to participate in the formation pathways of HAs [6, 30–32]. Thus, it is logical to hypothesize that trapping or scavenging of RCS may be a key inhibitory mechanism of HA formation for certain phytochemicals.

As a continuation of our mechanistic study, epigallocatechin gallate (EGCG) was chosen as the first object for detailed investigation. Since hydroxyl substituents are essential for phenolic compounds' free radical scavenging functionality, EGCG peracetate, in which all the hydroxyl groups are acetylated, was also tested to assess the degree to which the antioxidant activity of EGCG contributes to its effect on the formation of PhIP. Effective inhibition of PhIP formation by the peracetate indicates the existence of an antioxidant-independent mechanism. A wide range of chemical model reactions were subsequently performed to elucidate the probable mechanism involved. This included examination of the relative activity of EGCG and its peracetate in the generation of key PhIP intermediates as well as identification of reaction products arising from trapping of these intermediates by EGCG. A time-course study on the generation of these reaction products was also conducted to gain insight into the potential of EGCG as an effective inhibitor of PhIP formation in prolonged heating processes, which have been linked to enhanced HA-associated mutagenic activity in food products.

2 Materials and methods

2.1 Materials

Phenylalanine, glucose, phenylacetaldehyde, di(ethylene) glycol, diethyl ether and ammonium acetate were purchased from SigmaAldrich Company (St. Louis, MO, USA). Disodium hydrogen phosphate was from BDH Chemicals Ltd (Poole England). EGCG (98% purity) was obtained from Chromadex (Santa Ana, CA, USA). EGCG peracetate (92% purity) was prepared according the method of Kohri *et al*. [33]. PhIP standard was from Toronto Research Chemicals (Toronto, Canada). Propyl-sulfonic acid (PRS) Bond-Elut cartridges (500 mg), C-18 cartridges (100 mg), Bond-Elut reservoir and packing materials (diatomaceous earth) for solid-phase extraction were from Varian Inc. (Harbor City. CA, USA). All solvents used were of analytical grade and were obtained from BDH Laboratory Supplies (Poole, UK). The Reacti-Therm III heating module (model 18840) and the screw cap Tuf-Bond Teflon fitted glass reaction vials were purchased from Pierce (Rockford, IL, USA).

2.2 Model Maillard reaction

Effect of EGCG and EGCG peracetate on the formation of PhIP was examined according to the method described previously [26]. Their relative activity in the formation of PhIP Maillard intermediates was tested in chemical model systems containing 0.4 mmol phenylalanine, 0.2 mmol glucose (Glu), 0.4 mmol creatinine and 0.1 or 0.2 mmo1 EGCG or 0.1 mmol EGCG peracetate. For LC-MS identification of EGCG adduction products, model compositions are listed in Table 1. The reaction mixtures were dissolved in 20 mL 0.1 M phosphate buffer (pH 7.0) in crew cap Tuf-Bond Teflon fitted glass reaction vials (40-mL capacity) and heated in a Reacti-Therm III heating module at 128°C for 30 min. After heat treatment, the vials were cooled in a water bath for 40 min and then prepared for GC-MS or LC-MS analysis. For the investigation of the reaction between EGCG and phenylacetaldehyde, di(ethylene) glycol was used as the reaction medium because phenylacetaldehyde is insoluble in aqueous solvent systems. All other parameters were identical to those in the phenylalanine-glucose-creatinine models. At minimum, duplicate experiments were performed for all the model reactions.

2.3 Sample preparation

Sample preparation for testing the effect of EGCG and EGCG peracetate on the formation of PhIP was the same as in our previous study [26]. For analysis of volatile compounds, 15 mL from the 20-mL reaction mixtures was extracted with 8 mL diethyl ether [spiked with 80 μL *n*-dodecane (1 μL/mL), internal standard] with vortexing for 1 min. The two-phase samples were then centrifuged for

Table 1. Aqueous and di(ethylene) glycol chemical model reactions[a)]

Reactant	Reactant concentration (mM)									
	A	B	C	D	E	F	G	H	I	J
Phenylalanine	20		20	20	20	20	20		10	
Glucose			10	10	10	10		10		
Creatinine				20		20				
EGCG		5			5	5	5	5	5	5
[$^{13}C_2$]Phe									10	
Phenylacetaldehyde										20

a) Model reactions A – I were carried out in phosphate buffer (0.1 M, pH 7.0) and heated at 128°C for 30 min; model reaction J was in di(ethylene) glycol and heated at 128°C for 30 min.

10 min (8000 × *g*). The clear ether supernatant was then dried with anhydrous sodium sulfate, filtered and then subjected to GC-MS analysis.

For identification of adduction products, reaction mixtures from models listed in Table 1 were subjected to LC-MS analysis after a single syringe-driven filtering step without further sample processing. Samples from the phenylacetaldehyde models were diluted (610) in methanol before subjecting to LC-MS analysis.

2.4 GC-MS

The diethyl ether extracts were analyzed using an Agilent gas chromatograph (6890N) equipped with an autosampler (G2614A) and coupled to an Agilent mass spectrometer (5973N, EI mode). Separation was performed on a DB-Wax capillary column (30 m × 0.25 mm id, 0.25 μm film thickness). Analyses were carried out using the following parameters: 1 μL sample injected in splitless mode; inlet temperature, 200°C; column flow, 1 mL/min (He); temperature program, 40°C for 4 min, ramp at 5°C *per* min to 230°C and hold for 5 min; MS temperature, 230°C. Identification of phenylacetaldehyde was performed by comparing with mass spectrum and linear retention index of authentic standard analyzed under identical conditions. Percent inhibition of phenylacetaldehyde formation = TIC peak area of EGCG treatment / TIC peak area of control × 100. Peak area was adjusted by internal standard (*n*-dodecane).

2.5 LC-MS

All filtrates were analyzed on an LC-MS instrument equipped with an ESI source interfaced to a Finnigan LCQ-Deca XP mass spectrometer. LC was run on an Agilent HPLC system equipped with a degasser (G1379A), a quaternary pump (G1311A), a thermostatted autosampler (G1329A), and a diode array detector (G1315B). Separation of Maillard reaction products was carried out on an YMC-Pack ODS C-18 column (5 μm, 15 × 4.6 mm). The mobile phase comprised 10 mM ammonium acetate aqueous solution (solvent A) and ACN (B) of the following gradients: 0 min, 5% B:95% A; 35 min, 80% B:20% A; 37 min, 5% B:95% A; 50 min, 5% B:95% A. Effluent from the UV detector was split 4:1 with one part (200 μL/min) directed to the MS for spectrometric analysis and the remaining to waste. The MS conditions were as follows: negative ion mode, spray voltage 3.5 kV, scan range 120 – 1000 Da; capillary temperature 300°C. MS/MS analysis was set at *m/z* 559, and normalized collision energy at 30%.

2.6 Time course of direct trapping phenylacetaldehyde by EGCG

Phenylacetaldehyde and EGCG in molar ratio of 5:1 were first dissolved in di(ethylene) glycol to a final concentration of 125 and 25 mM, respectively. In each batch of experiments, samples were subjected to heat treatment for 15, 30, 60, 120 or 240 min. At each time point, samples were taken out from the heating module and immediately inserted into an ice-water mixture to stop further heat treatment. Subsequent sample preparation steps were as described in Section 2.3.

3 Results and discussion

3.1 Effect of EGCG and EGCG peracetate on PhIP formation

Previous studies have identified EGCG as one of the most potent natural inhibitors of PhIP formation [26, 29]. However, the mechanism of inhibition involved has not yet been clearly defined. With respect to inhibition of HA formation, most previous reports have attributed the inhibitory effect of phenolic compounds to their antioxidant/free radical scavenging capacity, which is ascribed to the hydrogen and electron donating capacity of their hydroxyl substituents [34, 35]. From a kinetic point of view, the large number of hydroxyl groups together with the pattern of their substitution grant EGCG extraordinary antioxidant activity that has been implicated in various beneficial biological activities such as protection against photodamage to skin cells, che-

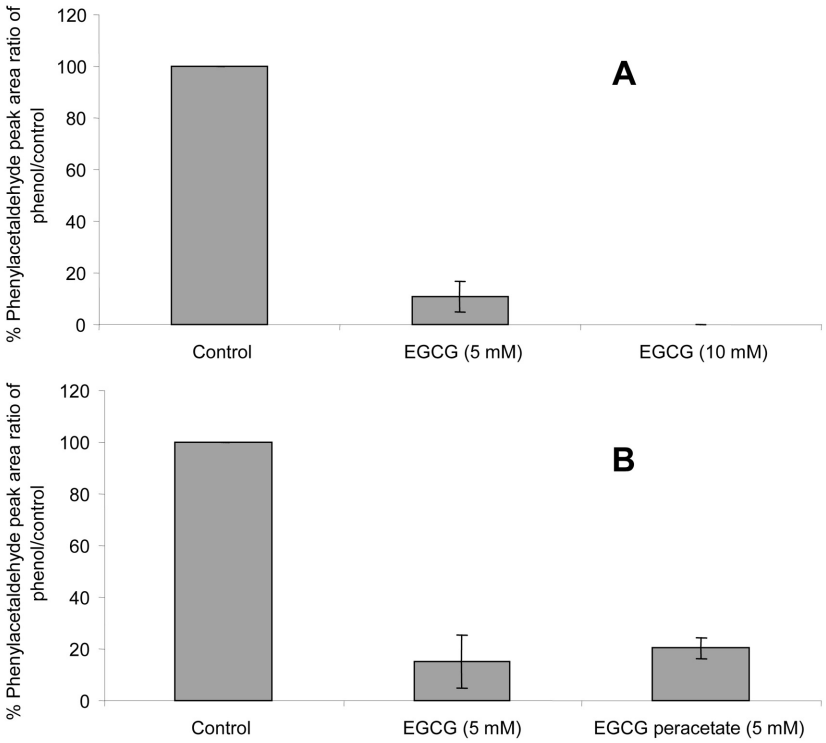

Figure 1. (A) Effect of EGCG on the formation of phenylacetaldehyde. (B) Relative activity of EGCG and EGCG peracetate in inhibiting the formation of phenylacetaldehyde. Reactions were performed in PhIP-producing aqueous chemical model systems at pH 7.0, 128°C for 30 min. *% inhibition = - TIC peak area of EGCG/EGCG peracetate treatment / TIC peak area of control × 100; peak area adjusted by internal standard (*n*-dodecane).

motherapeutic effects on colon cancer cells, cardiovascular protection, etc. [36 – 38].

Since hydroxyl substituents are essential for phenolic compounds' free radical scavenging functionality, assessment of the degree to which such functionality contributed to inhibitory activity of EGCG on PhIP formation can be accomplished by comparing its activity with that of EGCG peracetate, which has all its hydroxyl groups acetylated. Our result showed that both effectively reduced the formation of PhIP in chemical model systems. This suggested that an antioxidant-independent mechanism also played an important role in the inhibitory activity of EGCG. Since significant positive correlations between antioxidant and PhIP-inhibitory activity of phenolic compounds are still lacking [26, 29] and since both free radical-mediated mechanism and the Maillard reaction might be implicated in HA formation [6, 30, 31], it is possible that an alternative mechanism(s) could be mediated *via* scavenging/trapping of PhIP Maillard intermediates.

3.2 Effect of EGCG and EGCG peracetate on the formation of PhIP Maillard intermediates

To investigate the effect of EGCG on the formation of PhIP Maillard intermediates, GC-MS analysis was performed for samples from aqueous PhIP-producing models with or without the addition of EGCG. With the reaction conditions and sample preparation method adopted in the current study, phenylacetaldehyde was identified as the chief volatile compound formed. No other volatile related to the formation of PhIP, such as phenyethylamine [39, 40], was found. Quantitative analysis showed that EGCG in molar quantity as low as one-fourth that of phenylalanine was capable of suppressing the formation of phenylacetaldehyde by nearly 90% relative to the control (Fig. 1A). Doubling the level of EGCG reduced phenylacetaldehyde content to below detection limit. In addition, at equal molar concentration, EGCG was only slightly better than its peracetate in reducing the content of phenylacetaldehyde in model system (Fig. 1B). Heating of phenylalanine Maillard model can give rise to many degradation products, such as phenylacetaldehyde, phenylethylamine, styrol, phenylethanol and phenylacetic acid. Among these, only the first two have been demonstrated to form PhIP by reacting with creatinine [39]. The RCS phenylacetaldehyde is especially important since its yield of PhIP has been found to be ten times higher than that of phenylethylamine [39]. Therefore, it is probable that EGCG inhibits PhIP formation by interrupting the formation of these intermediate compounds from phenylalanine and/or by directly scavenging/trapping them after they were formed.

3.3 Identification of adducts formed from EGCG and phenylalanine degradation products

To verify our RCS-trapping hypothesis, LC-ESIMS^n analyses were conducted for samples from a wide range of model system reactions (Table 1) to identify adducts formed

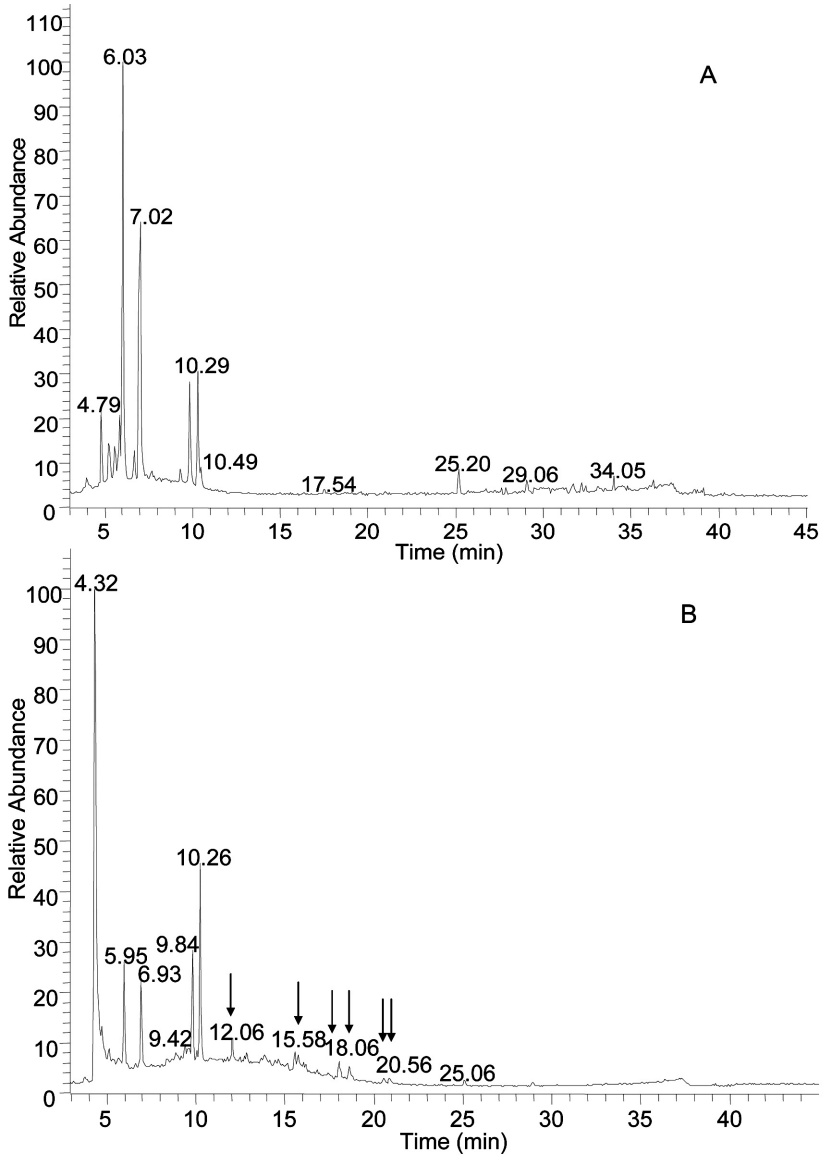

Figure 2. TIC chromatogram of samples from model B (A) and G (B). Peaks denoted with an arrow represent analytes with predicted molecular weight corresponding to adducts formed between EGCG and phenylalanine-degradation products and that were found only in models where both EGCG and phenylalanine were present.

between EGCG and phenylalanine degradation products. Samples in the present study were only processed by a single syringe-driven filtering step so that a wide spectrum of analytes could be retained for subsequent LC-MS analysis. LC-MS results indicated that this sample preparation procedure was adequate for achieving our analytical goals. All LC-MS analyses were operated in the negative ionization mode. Close examination and comparison of the LC-UV and MS TIC chromatograms revealed generation of a number of unique analytes with molecular weights corresponding to adducts formed between EGCG and phenylacetaldehyde in models containing phenylalanine plus EGCG with or without glucose or creatinine (models E, F, and G). That is, the predicted molecular weight of these adducts was larger than that of EGCG, but not equal to that of EGCG dimer or oligomer, EGCG oxidation products or phenylacetaldehyde oligomer. Judging from their appearance as distinct peaks in the TIC chromatograms, these analytes were presence in significant quantity in the corresponding model systems (Fig. 2). These characteristic analytes were not detected in models containing phenylalanine or EGCG alone (models A and B) and were also absent in models containing phenylalanine and glucose (model C), phenylalanine, glucose, and creatinine (model D) or EGCG and glucose (model H). Major analytes (especially those that were unique to models where both EGCG and phenylalanine were present) identified from model G as well as probable molecular compositions are presented in Table 2. Creatinine has been proposed to undergo aldol addition with phenylacetaldehyde in the formation of PhIP [35]. Surprisingly, it did not have obvious impact on the production of the aforementioned compounds, although its initial molar concentration in these models was much higher (4:1) than that of EGCG. This may imply that under such reaction condi-

Table 2. Major LC-MS analytes $[(M-H)]^-$ from model G

Model reaction	$[(M-H)]^-$ m/z (Rt, min)	Proposed molecular structure
EGCG + Phenylalanine	164 (4.32)	Phenylalanine
	457 (5.95, 6.93, 9.84, 10.26)	EGCG and its isomers
	559 (12.06, 12.85, 15.74, 18.06, 18.61, 20.56)	EGCG + Phenylacetaldehyde – H_2O
	743 (13.92)	2 EGCG + 2 Phenylacetaldehyde – 2 Gallic acid – $4H_2O$
	847 (15.58)	2 EGCG + Phenylacetaldehyde – Gallic acid – H_2O

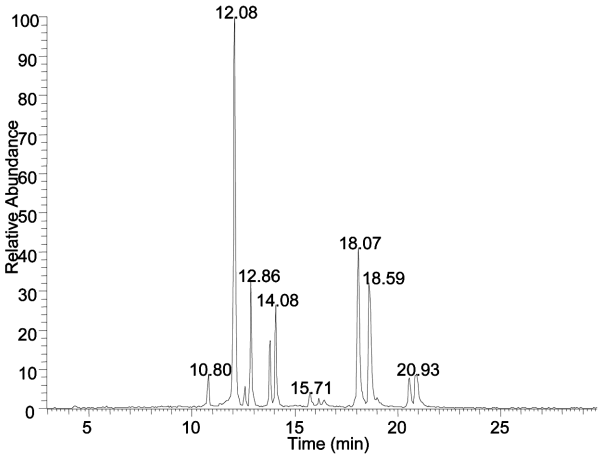

Figure 3. ESI-MS/MS chromatogram (parent *m/z* at 559) of sample from model G.

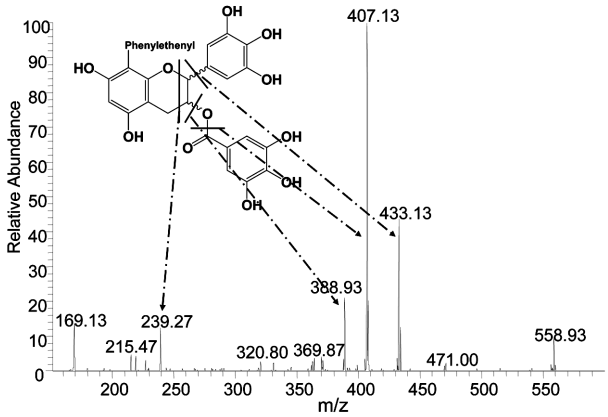

Figure 4. MS/MS spectrum of $[M-H]^-$ m/z 559 adduct.

tions, reactivity of EGCG towards phenylacetaldehyde was much higher than that of creatinine. The consumption of this RCS intermediate by EGCG would thus greatly reduce its availability for PhIP formation and as a result, effectively attenuates mutagenic activity of the reaction system concerned.

During the course of analysis, a selected set of samples were stored at $-4^{\circ}C$. Samples were analyzed weekly for four consecutive weeks under the same LC-MS conditions. Mass chromatograms showed that overall profile of adducts of interest as well as their ion intensity did not have much change, indicating very high stability of these final reaction products. This provided useful information for further study on the isolation and structural elucidation of these adducts and this is currently under way in our lab.

These findings tend to support the central role of phenylalanine in generation of the above characteristic adducts. Subsequently, isotope-labeling ($[^{13}C_2]$Phe) investigation (model I) was carried out to acquire more solid evidence. Mass spectra of most of the adduct analytes mentioned above displayed two isotopomers with equal ion intensity but differed by 1 Da. Some of them ($[M-H]^-$ m/z 743) displayed three isotopomers each differing by 1 Da and these could arise from the combination of two EGCG and two phenylacetaldehyde molecules that originated from two unlabeled, one $^{13}C_2$-labeled and one unlabeled and two $^{13}C_2$-labeled phenylalanine molecules, respectively, followed by removal of two Gallic acid and four water molecules. These observations strongly supported phenylalanine as a key participant in the proposed adduction reaction. The majority of the postulated adducts have a molecular weight of 560 (*m/z* 559). To gain an insight into the structural characteristics of these adducts, MS/MS was performed with parent *m/z* ratio set at 559. As displayed in Fig. 3, eight of the MW 560 adducts generated in models containing both EGCG and phenylalanine likely existed as four pair of isomers. CID of these analytes showed similar fragmentation patterns with diagnostic losses of 126, 152, 169, and 320 amu, respectively (Fig. 4). This fragmentation information together with literature data [41, 42] suggest that the MW 560 analytes likely resulted from electrophilic substitution of phenylacetaldehyde on the A ring (C-6 or C-8) of EGCG, followed by elimination of a water molecule to form phenylethenyl-EGCG. The probable pathways that may thus contribute to the inhibition of PhIP formation by EGCG are proposed in Fig. 5.

3.4 Capability of EGCG in directly trapping phenylacetaldehyde

Based on the predicted molecular weight of the analytes of interest mentioned above as well as their fragmentation behavior, the most important phenylalanine-derived fragment taking part in the proposed adduction reaction was likely phenylacetaldehyde. To further explore the capability of EGCG in direct trapping of phenylacetaldehyde, model

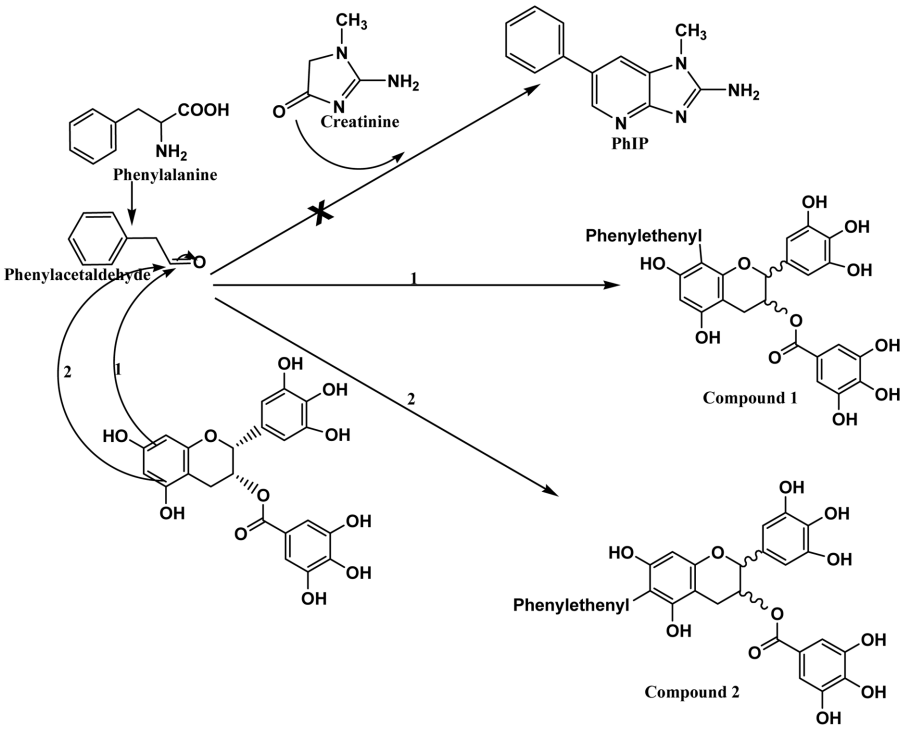

Figure 5. Postulated pathways for inhibitory activity of EGCG on PhIP formation.

reaction (model J) employing these two postulated adduction reactants was performed. Chromatograms extracted at *m/z* 559 showed only four major ion peaks. The retention time (Rt) and UV-absorption spectra of these four analytes completely matched with four of the adducts identified from models containing phenylalanine in place of phenylacetaldehyde. CID fragmentation behavior of these four *m/z* 559 adduct ions was also consistent with that of the corresponding adducts found in phenylalanine-containing models. As discussed in the previous section, a much larger number of adduction products were identified in models containing the parent amino acid phenylalanine (Table 2). This implied that adduction reaction in the EGCG-phenylacetaldehyde model with di(ethylene) glycol as the reaction medium had higher degree of selectivity. Alternatively, such reaction medium favored the existence of the adduction products mentioned above in particular configurations.

In addition to high temperature, prolonged heat treatment has also been linked to increased HA-associated mutagenic activity in food systems [6, 43 – 45]. Therefore, it would be ideal for inhibitors of HA formation to be capable of sustaining their inhibitory activity through these thermal processes. Consequently, a time-course study was carried out to assess the phenylacetaldehyde-trapping capability of EGCG. Figure 6 shows the UV-absorption spectra obtained from samples at selected durations of heat treatment. The concentration of target analytes increased while that of EGCG (or its isomer) (Rt. 9.8 min) decreased with time within the time frames investigated (15, 30, 60, 120, and 240 min). These analytes were not found in the control models (EGCG or phenylacetaldehyde alone). MS/MS analyses were performed for the analytes to determine their probable molecular composition and results showed that they correspond to adducts composing of EGCG and phenylacetaldehyde at different molar ratios. This indicated continuous generation and accumulation of these reaction products in the model systems. In other words, EGCG could be considered as an effective trapping agent of phenylacetaldehyde and such activity is sustainable in prolonged heating processes.

In recent years, there has been an increasing interest in understanding the chemistry of RCS. Under simulated physiological conditions (pH 7.4, 37°C), green tea catechins and black tea theaflavins were reported to effectively trap RCS such as glyoxal and methylglyoxal, suggesting good potential in protecting against diabetes complications [41, 42]. Our previous study also demonstrated that catechin dimers such as procyanidin B2 isolated from cinnamon bark could also be strong scavengers of RCS [46]. Another hot area of RCS research is associated with thermal processing of foods or simulated food models. The most extensively explored model has been the glycine-glucose model. Recent studies showed that epicatechin, epicatechin gallate and EGCG were capable of scavenging C2, C3 and C4 sugar fragments in aqueous glycine-glucose models heated at 125°C [47, 48]. As heat-induced degradation of glucose and glycine has been proposed to give rise to precursors for the formation of imidazole-quinoline- and imidazo-quinoxaline HAs [6, 32], depletion of these degradation products may be an important part of the inhibitory mechanism

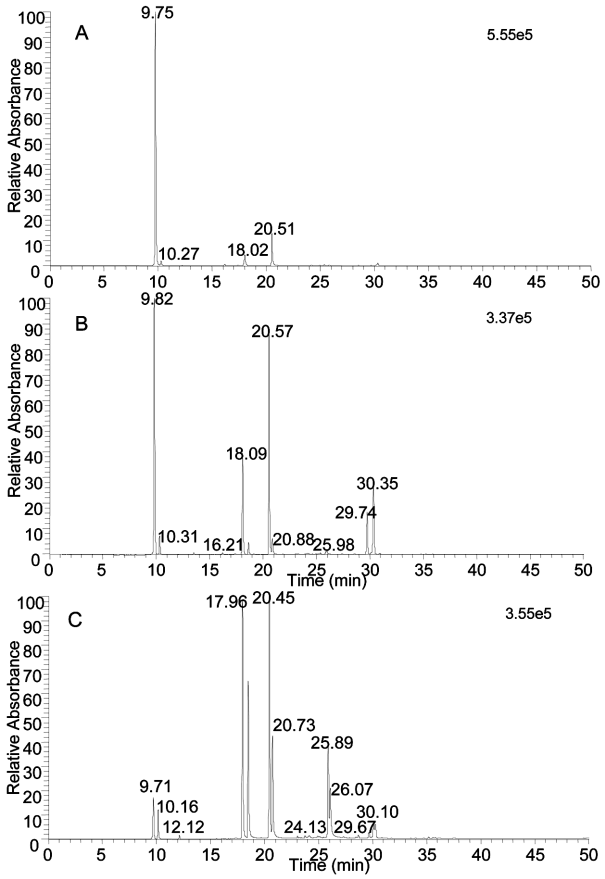

Figure 6. UV-absorption spectrum (315 nm) showing relative abundance of selected analytes in chemical models subjected to heat treatment at 128°C for 15 (A), 30 (B) and 240 min (C).

of these flavonoids against MeIQx formation, although strictly speaking models employed by these previous studies were not favorable surrogates for HA investigation in terms of precursors composition and precursors ratio [47 – 49]. Our findings, together with the literature, indicate that RCS trapping may be a key mechanism by which EGCG inhibits the formation of PhIP in the model system.

4 Concluding remarks

Findings from the present study showed that low levels of both EGCG and EGCG peracetate effectively inhibited the formation of PhIP and reduced the content of its key intermediate, phenylacetaldehyde, in PhIP-producing models. An isotope-labeling study and LC-MS analyses have offered strong evidence that EGCG is capable of forming many adduction products with phenylalanine or phenylalanine degradation products. The scavenging of phenylacetaldehyde, a key PhIP intermediate may thus lead to significant inhibition of PhIP formation. Although in the past tea was a rare ingredient of our daily diet, with the increasing popularity of incorporating diverse natural extracts claimed to have health benefits into our daily cuisine, tea extract rich in EGCG could be relevant to effective attenuation of HA-associated health risk, especially among populations of Oriental countries. Free radical scavenging has been proposed as the mechanism by which some phenolic antioxidants inhibit HA formation, but there has not yet been a clearly defined mechanistic link between these two parameters, especially when a large group of phenolic antioxidants are taken into consideration. The present study indicates that an antioxidant-independent mechanism exists that contributes to inhibitory activity of EGCG on PhIP formation. This mechanism may be mediated *via* scavenging of phenylacetaldehyde and/or inhibiting its formation from phenylalanine. These findings contribute significantly to our understanding of the lack of significant correlations between antioxidant and HA-inhibitory activity of certain phenols, providing useful information for further studies in the field of food chemistry and toxicology. They show that scavenging/trapping of amino acid-derived reactive species could be an effective approach to reducing the level of toxicants in food products that are associated with thermal processing. The finding that phenylacetaldehyde-scavenging activity of EGCG is sustainable through prolonged thermal treatment further supports EGCG as being a promising inhibitor of PhIP formation, as such heating processes have been associated with enhanced HA-related mutagenic activity in food products.

We thank the HKSAR Research Grand Council for financial support (Project HKU 7778/07M to Mingfu Wang)

The authors have declared no conflict of interest.

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