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Hydroxyl radical induced by lipid in Maillard reaction model system promotes diet-derived N_ε-carboxymethyllysine formation

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ABSTRACT

N_ε-carboxymethyllysine (CML) is commonly found in food, and is considered as a potential hazard to human health. However, the effect of lipids on CML formation in Maillard reaction is still not clarified. In this study, the content of diet-derived CML and its key intermediates, epsilon-fructoselysine (FL) and glyoxal (GO), is determined with high performance liquid chromatography mass spectrum (HPLC-MS) in model system containing lipid compounds. According to the results, hydroxyl radical ($OH^{\cdot}$) induced by Fenton reagent can promote the three pathways of CML formation. Moreover, in the Maillard reaction system, linoleic acid (Lin), oleic acid (Ole) and glycerol trioleate (Tri) can induce more $OH^{\cdot}$, which promotes CML formation. Their level of promoting CML formation is in the order of Ole > Lin > Tri. On the contrary, glycerol (Gly) can scavenge $OH^{\cdot}$, which inhibit the CML formation. Finally, it is proved that FL content and GO content decreases with heating time in model system, while CML content increases with heating time. Thus, it is concluded that in the Maillard reaction system lipids can induce more $OH^{\cdot}$, which promotes the conversion from FL and GO to CML. Our research may contribute to the development of inhibitory methods for diet-derived CML by scavenging $OH^{\cdot}$.

1. Introduction

Advanced glycation end products (AGEs) are formed in the late stage of the Maillard reaction between carbonyl groups (carbohydrates and lipids) and amino groups (proteins and nucleic acids) (Singh et al., 2001). AGEs include N_ε-carboxymethyllysine (CML), N_ε-carboxyethyllysine, pentosidine, vesperlysine, pyrraline, imidazolone, etc. (Thornalley, 2005). It was shown that AGEs existed in tissue (Schalkwijk et al., 2004), plasma (Hartog et al., 2007), urine (Matsunaga et al., 2005) and tears (Zhao et al., 2010) of diabetic complication patients, and the content of AGEs in human body increased with age. In addition, food is a great source of AGEs, and the adverse effect of diet-derived AGEs on human health was proved by many animal and clinical experiments (Sebekova et al., 2003; Lin et al., 2002; Bartling et al., 2007; Semba et al., 2009; Uribarri et al., 2003a, 2003b).

CML is deemed as a typical example of AGEs, and its chemical structure and determination methods were reported. (Thorpe and Baynes, 2002). Recently, food scientists were surprised to find that CML was widely distributed in diet, especially in oil foods. Goldberg et al. (2004) compared the content of CML in 250 kinds of food classified by oil, protein and carbohydrate, and found that oil food had the highest content of CML. Uribarri et al. (2010) expanded the available CML database with the content of 546 kinds of commom food, and further confirmed the previous finding. Srey et al. (2010) studied the effect of cooking oil on CML formation in model food. They found that cakes prepared with oil had more CML than those prepared with sucrose. On the contrary, Hull et al. (2012) determined the content of CML in 257 kinds of food with high performance liquid chromatography mass spectrum (UPLC-MS) instead of enzyme-linked immunosorbent assay, and found that the content of CML in oil food was not always higher than that in other foods. It depended on data representation format. Lima et al. (2010) compared casein-glucose model system and casein-glucose-linolenic acid model system under the condition of being heated at 95 °C for 8 h. They found that CML formed in the latter one was less than that of the former one, suggesting a negative effect of oil on CML formation. Although there still remains controversy about whether oil promotes CML formation in food, there

Abbreviations: CML, N_ε-carboxymethyllysine; FL, epsilon-fructoselysine; GO, glyoxal; HPLC-MS, high performance liquid chromatography mass spectrum; OH, hydroxyl radical; Lin, linoleic; Ole, oleic acid; Tri, glycerol trioleate; Gly, glycerol; Lys, alpha-Boc-lysine; Glu, glucose; AGEs, advanced glycation end products; DAN, 2,3-Diaminonaphthalene; SA, salicylic acid; 2,3-DHBA, 2,3-dihydroxy benzoic acid; 2,4-DHBA, 2,4-dihydroxy benzoic acid; 2,5-DHBA, 2,5-dihydroxy benzoic acid; DMF, 1-deoxy-1-morpholino-D-fructose; NBT, nitroblue tetrazolium; SIR, Single Ion Recording.

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are few reports on the mechanism of how lipids affect CML formation in Maillard reaction system.

CML formation is affected by many components in real food system. Choosing a simple model system is certainly a useful approach to reveal the mechanism effectively. To reveal the mechanism of CML formation, previous studies have mainly focused on intermediates and pathways. Two intermediates, epsilon-fructoselysine (FL) and glyoxal (GO), are involved in the three pathways of CML formation as shown in Fig. 1 (Erbersdobler and Somoza, 2007; Ferreira et al., 2003). In Autoxidative glycosylation pathway, GO derived from glucose (Glu) oxidation reacts with alpha-Boc-lysine (Lys) to form CML (Wolff and Dean, 1987). In Namiki pathway, GO derived from Schiff's base reacts with Lys to form CML (Glomb and Monnier, 1995). In Glycosylation pathway, Schiff's base undergoes Amadori rearrangement to form FL, which is then oxidized to form CML (Ahmed et al., 1986).

Although there are many researches on intermediates and pathways, the effect of free radical on them remains unclear. Nagai et al. (1997) confirmed that hydroxyl radical (OH·) induced by Fenton reagent could promote the conversion from FL to CML in Glycosylation pathway. However, the effect of OH· on Autoxidative glycosylation pathway and Namiki pathway remains unclear. In other words, we wonder whether OH· has promoting effect on the reaction between Lys and GO to form CML in Autoxidative glycosylation pathway and Namiki pathway.

Maillard reaction occurs in vivo at 37 °C for several decades. In contrast, Maillard reaction in food processing results from 100 to 250 °C heating for short periods. The aim of our study is to investigate, in Maillard reaction model system at 100 °C, the effect of lipids on CML formation from the angle of OH·. Firstly, the effect of OH·, induced by Fenton reagent, on Lys + Glu model system and Lys + GO model system was investigated to illustrate that OH· could promote the three pathways of CML formation. Secondly, the OH· formation in Lys + Glu + Lipid model system and Lys + GO + Lipid model system was proved. Thirdly, oleic acid (Ole), linoleic acid (Lin) (both used as representative for common unsaturated fatty acids of triglyceride in oil), glycerol trioleate (Tri, used as a representative for triglyceride in oil) and Gly were added to Lys + Glu model system and Lys + GO model system respectively. The effect of lipids on CML formation was investigated by determining the content of OH·, CML, FL and GO. Our research may contribute to the development of inhibitory methods for diet-derived CML by scavenging OH·.

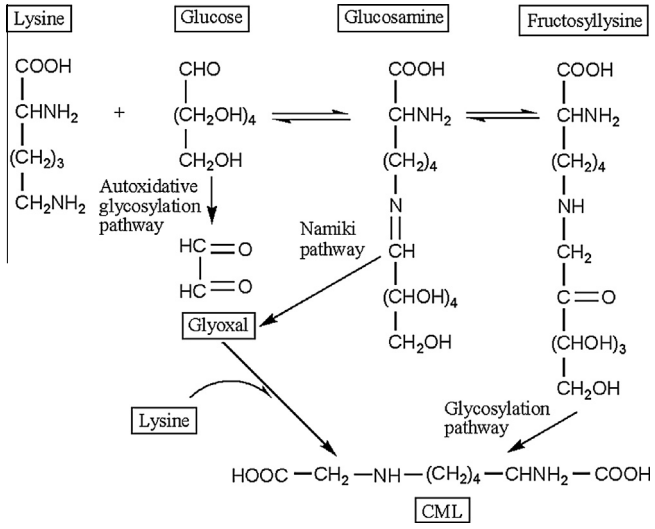

Fig. 1. Three pathways of CML formation.

2. Materials and methods

2.1. Materials

CML (98%) was purchased from Toronto Research Chemicals (Canada). GO (40% aqueous solution), H_2O_2 (30% aqueous solution), 2,3-Diaminonaphthalene (DAN), Lin (99%), Ole (99%), Tri (99%), salicylic acid (SA), 2,3-dihydroxy benzoic acid (2,3-DHBA), 2,4-dihydroxy benzoic acid (2,4-DHBA), 2,5-dihydroxy benzoic acid (2,5-DHBA) were purchased from Shanghai Crystal Pure Industrial Company Limited (China). Gly and 1-deoxy-1-morpholino-D-fructose (DMF) were purchased from Sigma Company (USA). Glu, Lys, methanol, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium carbonate, sodium bicarbonate, nitroblue tetrazolium (NBT), ferrous chloride ($FeCl_2$) and diethyl ether were purchased from Sinopharm Chemical Reagent Company Limited (China). All reagents were of analytical grade.

2.2. Preparation of model system

Lys + Glu + Fenton model system: phosphate buffer (0.2 mol/L, pH = 7.0) solution containing Lys (0.1 mol/L), Glu (0.1 mol/L), $FeCl_2$ (0.001 mol/L) and H_2O_2 respectively of 0 mol/L, 0.0001 mol/L, 0.001 mol/L and 0.01 mol/L were aliquoted into the same glass vials. Each glass vial containing 1 mL solution was then sealed and placed in a water bath at 40 °C for 1 h.

Lys + GO + Fenton model system: phosphate buffer (0.2 mol/L, pH = 7.0) solution containing Lys (0.1 mol/L), GO (0.1 mol/L), $FeCl_2$ (0.001 mol/L) and H_2O_2 respectively of 0 mol/L, 0.0001 mol/L, 0.0005 mol/L and 0.001 mol/L were aliquoted into the same glass vials. Each glass vial containing 1 mL solution was then sealed and placed in a water bath at 40 °C for 1 h.

Lys + Glu model system: phosphate buffer (0.2 mol/L, pH = 7.0) solution containing Lys (0.1 mol/L) and Glu (0.1 mol/L) was aliquoted into the same glass vials. Each glass vial containing 1 mL solution was then sealed and placed in a water bath at 100 °C for a set time.

Lys + Glu + Gly model system: phosphate buffer (0.2 mol/L, pH = 7.0) solution containing Lys (0.1 mol/L), Glu (0.1 mol/L) and Gly (0.1 mol/L) was aliquoted into the same glass vials. Each glass vial containing 1 mL solution was then sealed and placed in a water bath at 100 °C for a set time.

Lys + Glu + Lipid model system: Lin, Ole or Tri was dissolved in diethyl ether and aliquoted into the same glass vials, each one containing 0.1 mmol lipid. After diethyl ether was volatilized with nitrogen gas, phosphate buffer (0.2 mol/L, pH = 7.0) containing Lys (0.1 mol/L) and Glu (0.1 mol/L) was aliquoted into the same glass vials. Each glass vial containing 1 mL solution was then sealed and placed in a water bath at 100 °C for a set time.

The model systems after being heated were cooled in an ice bath and then analyzed with HPLC-MS (Waters 1525, Waters Micromass ZQ, USA).

2.3. Determination of synthesized FL

A standard curve was made for DMF as describe previously (Baker et al., 1994) with some modifications. 2 mL carbonate buffer (pH = 10.4) was added with a certain amount of DMF and 100 μL NBT solution (5 mmol/L). It was heated at 37 °C for 5 min and cooled in an ice bath. Subsequently, it was detected with ultraviolet visible spectrophotometer (VARIAN, USA) at 550 nm for absorbance.

A previously described determination method of synthesized FL (Horvat and Jakas, 2004) was applied with some modifications One milliliter water solution containing Lys (0.1 mol/L) and Glu (0.1 mol/L) was sealed in a glass vial, and heated at 100 °C for 0.5 h. Then it was cooled in an ice bath, and diluted 5 times with distilled water. One milliliter of this solution was passed through a conditioned C_{18} solid-phase extraction (SPE) column (2000 mg/12 mL, Agela Technologies, China), which was then eluted with 9 mL carbonate buffer (pH = 10.4). One hundred μL NBT solution (5 mmol/L) was added to 2 mL solution from 10 mL evenly mixed eluent. After being heated in a water bath at 37 °C for 5 min, its content was determined with ultraviolet visible spectrophotometerby at 550 nm by external reference method with standard curve made from DMF. The content of synthesized FL was 236 μg/mL.

2.4. Determination of CML and FL

A previously described method (Yeboah and Yaylayan, 2001; Lapolla et al., 2001; Gonzalez-Reche et al., 2006; Teerlink et al., 2004) was applied with some modifications. After being heated, 1 mL model system solution in the glass vial was cooled in an ice bath and diluted 10 times with distilled water. One milliliter solution of this dilution was passed through the conditioned C_{18} SPE column, which was then eluted with 9 mL methanol–water (10:90, v/v). One milliliter solution from 10 mL evenly mixed eluent was determined with HPLC-MS. Separation was conducted on an Atlantis C_{18} (5 μm, 4.6 × 150 mm, Waters, America) analytical column. Mobile phase was methanol–water (10:90, v/v). Flow rate was 0.5 mL/min. Mass spectrum conditions were as follow: capillary voltage was 3.0 kV, cone voltage was 20 V, ion source temperature was 100 °C, and desolution temperature was 300 °C. Single Ion Recording (SIR) was conducted by operating the MS in ESI mode, and *m/z* for CML and FL was 205 (M^+) and 309 (M^+). Injection volume was