Glyoxal derived from triglyceride participating in diet-derived Nε-carboxymethyllysine formation

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ABSTRACT

Diet-derived Nε-carboxymethyllysine (CML) is a potential hazard to human health. In this study, the content of CML and its intermediates, fructoselysine and glyoxal (GO), are determined with HPLC–MS in ^{13}C labeled isotope lipid food model system. It has been testified that GO, derived from linoleic acid (Lin), oleic acid (Ole) and glycerol trioleate (Tri) oxidation, participated in CML formation, marking a new pathway of CML formation. Moreover, five vegetable oils are proved to function favorably towards CML formation CML formation. Analysis on the fatty acids of the five oils indicates a positive effect of unsaturated fatty acids (UFA) on CML formation. Furthermore, the content of CML in milk increases with the increase of cream. Thus, one possible explanation for the high CML content in oil diet is that triglyceride with more UFA is capable of being oxidized to more GO which will participate in CML formation. Our research will contribute to the final solution of controlling the formation of diet-derived CML by inhibiting lipids oxidation and GO formation.

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1. Introduction

Advanced glycation end products (AGEs) are formed in the late stage of Maillard reaction. AGEs comprise carboxymethyllysine (CML), Nε-carboxyethyllysine, pentosidine, vesperlysine, pyrraline, imidazolone, etc. AGEs exist in lesion tissue (Schalkwijk, Baidoshvili, Stehouwer, Van Hinsbergh, & Niessen, 2004), plasma (Hartog et al., 2007; Kanauchi, Tsujimoto, & Hashimoto, 2001), urine (Matsunaga et al., 2005) and tear (Zhao et al., 2012) of diabetic complication patients. Most of these studies are concentrated on endogenous AGEs. Some animals and clinical experiments proved that diet-derived AGEs had adverse effects on human health (Bartling et al., 2007; Grasa, Calvo, Delgado-Andrade, & Navarro, 2012; Lin et al., 2002; Lin et al., 2003; Ruhs et al., 2007; Sebekova, Fais, Hofmann, Schinzel, & Heidland, 2003; Sebekova & Somoza, 2007; Semba et al., 2009; Uribarri et al., 2003a,b; Zheng et al., 2002) although the molecular mechanism of diet-derived AGEs on human health still remains unclear.

CML has long been deemed as a typical example of diet-derived and endogenous AGEs, and its chemical structure and analytical determination are well established. In recent years, the content of diet-derived CML is found to be high in foods, particularly in oil diet. Goldberg et al. (2004) compared the amount of CML in 250 kinds of foods and found that oil diet stood out with more CML. Uribarri et al. (2010) set up an available CML database with 546 kinds of common foods and further confirmed that food containing

oil were more prone to forming CML. Srey et al. (2010) studied the effect of cooking oil on CML formation in model food, and found that the more cooking oil was used in cake, the more CML was produced. On the contrary, Lima, Assar, and Ames (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, and found that CML produced in the latter one is less than that of the former one, suggesting a negative effect of oil on CML formation. Although former studies have reported on the macroscopic effect of oil on CML formation, there are few reports on the molecular mechanism describing the participation of oil in CML formation.

To reveal the mechanism of CML formation, previous studies on CML formation mainly focused on pathways and intermediates. Fructoselysine (FL) and glyoxal (GO) are key intermediates for the three pathways of CML formation (Erbersdobler & Somoza, 2007; Ferreira, Freire, & Voit, 2003; Thorpe & Baynes, 2002) including (i) autoxidative glycosylation pathway where GO derived from glucose oxidation reacts with lysine (Lys) to form CML (Wolff & Dean, 1987), (ii) Namiki pathway where GO derived from Schiff's base decomposition reacts with Lys to form CML (Glomb & Monnier, 1995) and (iii) glycosylation pathway where Schiff's base undergoes Amadori rearrangement to form FL which then oxidized to form CML (Ahmed, Thorpe, & Baynes, 1986). However, little attention has been paid to the participation of intermediates derived from oil in CML formation. Research indicated that lipids in food which underwent severe oxidation in high temperature heating will produce GO as precursors for diet-derived CML (Shibamoto, 2006). Fujioka and Shibamoto (2004) treated dietary oils: tuna, salmon, cod liver, soybean, olive, and corn oils in both accelerated storage condition and cooking condition, and the result demonstrated that fish oils with polyunsaturated

fatty acid (PUFA) produced more GO than vegetable oils did. Thus, we wonder whether GO derived from lipids oxidation participates in CML formation and whether the types of fatty acid have effect on CML formation.

This study aims at investigating whether GO derived from lipids oxidation participates in the formation of diet-derived CML. Three different systems with increasing complexity were investigated, ranging from ^{13}C labeled isotope Lys + glucose (Glu) + Lipid model system, Lys + Glu + Oil model system to the real food system of milk, so as to reveal molecular mechanism for the high content of CML in oil diet. Our research will contribute to the control over the formation of diet-derived CML.

2. Materials and methods

2.1. Materials

CML (98%) was purchased from Toronto Research Chemicals (Canada). GO (40% aqueous solution), 2,3-diaminonaphthalene (DAN), linoleic acid (Lin, 99%), ole acid (Ole, 99%) and glycerol trioleate (Tri, 99%) were purchased from Shanghai Crystal Pure Industrial Company Limited (China). Glu (1,2,3,4,5,6-^{13}C$_6$, 99%) was purchased from Cambridge Isotope Laboratories. Lys, Glu, methanol, sodium dihydrogen phosphate, disodium hydrogen phosphate, diethyl ether, sodium borohydride and sodium tetraborate were purchased from Sinopharm Chemical Reagent Company Limited (China). All reagents were of analytical grade. Soybean oil (Soybean), corn oil (Corn), olive oil (Olive), palm oil (Palm) and rape oil (Rape) were all refined oils offered by Shenzhen Jingyi Technology Limited Company without adding any antioxidants. Skim milk (SM, 3 g protein, 5 g lactose and 0 g milk fat per 100 g milk), low lactose milk (LLM, 3 g protein, 0.5 g lactose, 4.5 g Glu, 4.5 g galactose and 0 g milk fat per 100 g milk) and cream oil (Cream, 35.5 g milk fat per 100 g oil) were purchased from local supermarkets.

2.2. Preparation of Lys + Glu + Lipid model system and Lys + Glu + Oil model system

2.2.1. Lys + Glu + Lipid model system

Lin, Ole or Tri were dissolved in diethyl ether and aliquoted into glass vials, each containing 0.1 mmol. 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 (1,2,3,4,5,6-^{13}C$_6$, 0.1 mol/L) was aliquoted into the same glass vials, each one containing 1 mL solution, which were then sealed and placed in water bath at 100 °C for a set time.

2.2.2. Lys + Glu + Oil model system

Soybean, Corn, Olive, Palm or Rape were dissolved in diethyl ether and aliquoted into glass vials, each one containing 0.1 mmol oil (all oil molecular weight was calculated as 885 g/mol). 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 one containing 1 mL solution, which were then sealed and placed in water bath at 100 °C for a set time.

The model systems above were cooled instantly in ice water bath after being heated for a set time, and then the system was determined with HPLC–MS (Waters 1525, Waters Micromass ZQ, America).

2.3. Determination of fatty acids proportion in vegetable oils

Five oils were esterified with methanol and sulfuric acid. Fifty milligrams of oil was added to 2 mL methanol (containing sulfuric acid in volume fraction of 1%) and the solution was heated at 70 °C for 1 h. After 2 mL hexane was added, distilled water was added till the solution reached the bottleneck, then the solution was shaken up. The upper layer hexane liquid was taken out. The lower layer liquid was extracted with 1 mL hexane that was then merged with former hexane. Afterwards, samples were analyzed by GC/MS (Agilent, 6890 N, America). GC conditions were as follows: separation was performed on a capillary column (Agilent, DB-23, America). Helium was the carrier gas at flow rate of 1.0 mL/min. Temperature program was as follows: (1) 130 °C for 1 min, (2) increase to 220 °C at 5 °C/min, and (3) 220 °C for 5 min. The injection volume was 10 μL. MS conditions were as follows: full scan monitoring mode (m/z 33–500). Source temperature was set at 230 °C.

2.4. Determination of CML in Lys + Glu + Lipid and Lys + Glu + Oil model system

Applied method referred to the method described previously (Gonzalez-Reche, Kucharczyk, Musiol, & Kraus, 2006; Lapolla, Fedele, & Traldi, 2001; Teerlink, Barto, Ten, & Schalkwijk, 2004; Yeboah & Yaylayan, 2001) with modification. After being heated, 1 mL model system solution in the glass vial was cooled in ice bath and diluted 10 times with distilled water. One milliliter of this dilution was passed through conditioned C$_{18}$ SPE column (2000 mg/12 mL, Agela Technologies, China), which was then eluted with 9 mL methanol–water (10:90, v/v). One milliliter solution from the 10 mL evenly mixed eluent from the last step was determined with HPLC–MS. The liquid phase conditions were as follows: separations were conducted on an Atlantis C$_{18}$ (5 μm, 4.6×150 mm) analytical column. The mobile phase was methanol–water (10:90, v/v). The flow rate was 0.5 mL/min. The mass spectrum conditions were as follows: the capillary voltage was 3.0 kV, the cone voltage was 20 V, the ion source temperature was 100 °C and the desolution temperature was 300 °C. Single ion recording (SIR) was conducted by operating the MS in ESI mode and m/z for CML and CML (1,2-^{13}C$_2$) were 205 (M$^+$) and 207 (M$^+$). The injection volume was 10 μL. Data were analyzed using Empower software supplied by Waters. Analytes were quantified by reference to an external standard calibration curve by plotting MS area ratio against standard substance concentration. The retention time of CML and CML (1,2-^{13}C$_2$) was detected to be 3.5 min.

Contribution rates of Lin, Ole and Tri to CML formation were defined as molar percentages of CML derived from Lin, Ole or Tri to the sum of CML derived from Lin, Ole or Tri and CML (1,2-^{13}C$_2$) derived from Glu (1,2,3,4,5,6-^{13}C$_6$). Contribution rate of Glu to CML formation was defined as molar percentage of CML (1,2-^{13}C$_2$) derived from Glu (1,2,3,4,5,6-^{13}C$_6$) to the sum of CML derived from Lin, Ole or Tri and CML (1,2-^{13}C$_2$) derived from Glu (1,2,3,4,5,6-^{13}C$_6$).

2.5. Determination of GO in Lys + Glu + Lipid model system

Applied method referred to a method described previously (Han et al., 2007; Neng, Cordeiro, Freire, & Nogueira, 2007; Odani, Shinzato, Matsumoto, Usami, & Maeda, 1999; Randell, Vasdev, & Gill, 2005) with modification. After being heated, 1 mL model system solution in glass vial was cooled in ice bath and diluted 5 times with distilled water. One milliliter of this dilution was passed through conditioned C$_{18}$ SPE column, and was then eluted with 4 mL water. One hundred microliter DAN solution (0.1%) was added to 1 mL solution from 5 mL evenly mixed eluent. The sample was derivatized at 4 °C for 24 h. The reaction mixture was extracted with 4 mL of ethyl acetate, 3 mL upper layer of which was taken out and dried under nitrogen atmosphere. The dried extract was reconstituted with 200 μL methanol for HPLC–MS analysis. The liquid phase conditions were as follows: separations were conducted on a Symmetry C$_{18}$ (5 μm, 3.9×150 mm) analytical column. The mobile phase is 100% methanol and the flow rate was 0.5 mL/min. The mass spectrum conditions were as follows: the capillary voltage was 3.0 kV, the cone voltage was 20 V, the ion source temperature was 100 °C and the desolution temperature was 300 °C. SIR was conducted by operating the MS in ESI mode and m/z for GO-DAN and GO (1,2-^{13}C$_2$)-DAN were 181 (M^{23+}) and 183 (M^{23+}). The injection