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Effect of glycation derived from α-dicarbonyl compounds on the *in vitro* digestibility of β-casein and β-lactoglobulin: A model study with glyoxal, methylglyoxal and butanedione

Di Zhao[a], Thao T. Le[d], Lotte Bach Larsen[d], Lin Li[a,b,c], Dan Qin[a], Guoying Su[a], Bing Li[a,b,*]

[a] College of Food Science and Engineering, South China University of Technology, 381 Wushan Road, Guangzhou 510640, China
[b] Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, 381 Wushan Road, Guangzhou 510640, China
[c] School of Chemical Engineering and Energy Technology, Dongguan University of Technology, College Road 1, Dongguan 523808, China
[d] Department of Food Science, Aarhus University, Blichers Allé 20, Tjele 8830, Denmark

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ABSTRACT

α-Dicarbonyl compounds, which are widely found in common consumed food, are one of the precursors of advanced glycation end products (AGEs). In this study, the effect of glycation derived from glyoxal (GO), methylglyoxal (MGO) or butanedione (BU) on the *in vitro* digestibility of β-casein (β-CN) and β-lactoglobulin (β-Lg) was investigated. Glycation from α-dicarbonyl compounds reduced the *in vitro* digestibility of studied proteins in both gastric and intestinal stage. In addition, glycation substantially altered the peptides released through gastric and gastrointestinal digestion, as detected by liquid chromatography electrospray-ionization tandem mass spectrometry (LC-ESI-MS/MS). Crosslinked glycation structures derived from BU considerably reduced the sensitivity of glycated β-Lg towards digestive proteases, albeit to a lesser degree in glycated β-CN due to its intrinsic unordered structure. By contrast, non-crosslinked AGEs that formed adjacent to enzymatic cleavage sites did not block the enzymatic reaction in several cases, as evidenced by the corresponding digested peptides modified with glycation structures. These findings expand our understanding of the nutritional influence of α-dicarbonyl compounds and health impact of relevant dietary AGEs.

1. Introduction

Maillard reaction or glycation contain a series of reactions that occur in food industry. These complex chemical reactions can be divided into early (carbonyl-amine condensation and Amadori rearrangement), advanced (sugar fragmentation and strecker degradation) and final phase (heterocyclization and melanoidins formation) (Hellwig & Henle, 2014). Advanced glycation end products (AGEs) are lysine (Lys)-, arginine (Arg)- or N-terminus derived glycation structures that are generated in the advanced stage of the Maillard reaction through Amadori pathway, dicarbonyl pathway or radical reaction (Nguyen, van der Fels-Klerx, & van Boekel, 2016; Poulsen et al., 2013). AGEs, formed *in vivo* are involved in the vascular complications of diabetes, uraemia, and aging, (Murata et al., 1997; Nagaraj, Linetsky, & Stitt, 2012; Schleicher, Wagner, & Nerlich, 1997). AGEs present in ingested food (dietary AGEs) contribute significantly to the AGEs level in serum and organs after being absorbed in small intestine (Li et al., 2015; Uribarri et al., 2005). Several animal and clinical studies reported that the absorbed dietary AGEs are possible hazard because of their ability to combine with their receptors (RAGEs) *in vivo*, even though more evidences regarding their bioavailability and metabolism *in vivo* are still needed (Grossin et al., 2015; Poulsen et al., 2013).

α-Dicarbonyl compounds are a class of compounds with two ortho carbonyl groups formed during caramelization (sugar fragmentation) or the advanced stage of the Maillard reaction (fragments of Amadori compounds or glyceraldehyde alkylimine). 3-Deoxyglucosone (3-DG), 3-deoxygalactosone (3-DGal), glyoxal (MGO), methylglyoxal (GO) and butanedione (BU), are the major α-dicarbonyl compounds derived from glycoxidation (Hellwig, Degen, & Henle, 2010; Hollnagel & Kroh, 1998; Thornalley, 2005). Oil oxidation is another important source of α-dicarbonyl compounds, mainly generating BU, GO and MGO (Jiang, Hengel, Pan, Seiber, & Shibamoto, 2013). These α-dicarbonyl compounds are widely detected in commonly consumed foods, such as sweets, honey, dairy, jams and bakery products (Degen, Hellwig, & Henle, 2012), and their contents increase during heat treatment in food processing (Jiang et al., 2013; Kokkinidou & Peterson, 2014).

* Corresponding author at: College of Food Science and Engineering, South China University of Technology, 381 Wushan Road, Guangzhou 510640, China.
E-mail address: lcbingli@scut.edu.cn (B. Li).

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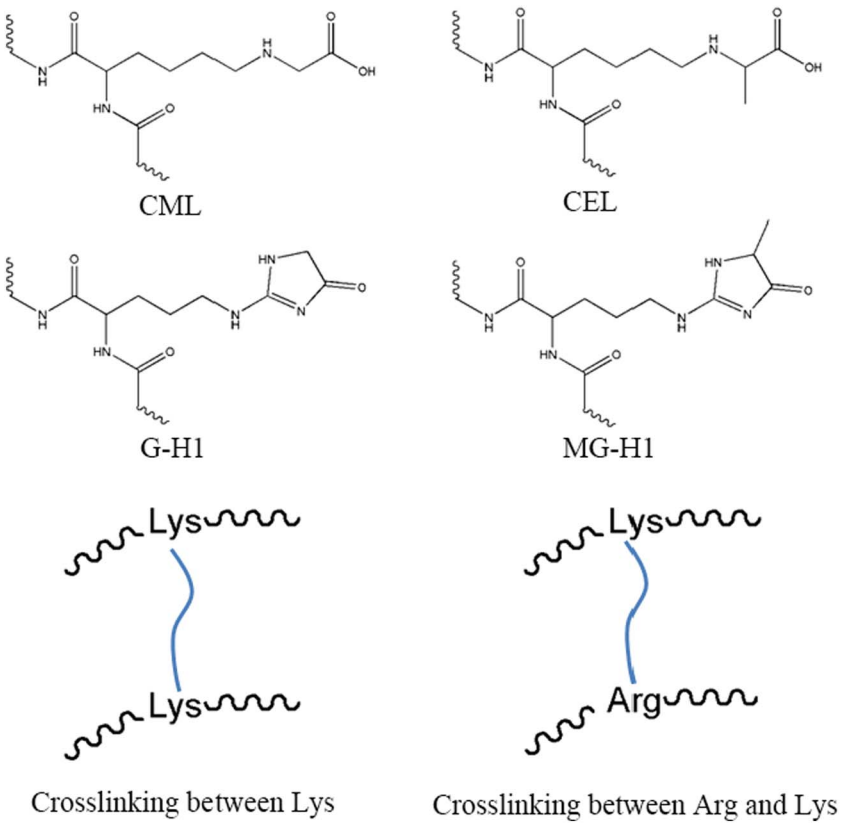

Fig. 1. Chemical structures of crosslinked and non-crosslinked MRPs discussed in this study.

After being generated in food processing or storage, these compounds are prone to react with side chains of Lys and Arg residues of dietary proteins and generate AGEs. GO has been proposed to be important precursor of *N* (ε)-carboxymethyllysine (CML, Fig. 1), GO hydroimidazolone (G-H, Fig. 1) and glyoxal lysine dimer (GOLD), whereas MGO is the precursor of *N* (ε)-carboxyethylline (CEL, Fig. 1), MGO hydroimidazolone (MG-H1, Fig. 1) and methylglyoxal lysine dimmer (MOLD) (Glomb & Lang, 2001; Lederer & Klaiber, 1999). Reaction between BU and side chain of Arg was reported to produce hydroimidazolone compounds which have similar structure to G-H and MG-H, which may be related to the etiology of obliterative bronchiolitis (Mathews, Watson, Snyder, Burgess, & Morgan, 2010).

Glycated proteins are the major origin of dietary AGEs. Before reaching the small intestine, AGEs bound to protein (protein-AGEs) will be enzymatically hydrolyzed into absorbable or unabsorbable fractions during their passage through the gastrointestinal tract. Therefore, digestion of protein-AGEs is closely related to their absorption in the small intestine, and was studied in this work. Considering the ubiquity of α-dicarbonyl compounds in processed food and their role in AGEs formation, investigating the effect of glycation derived from α-carbonyl compounds on protein digestiblity should elucidate the nutritional impact of these compounds and the health effect of α-dicarbonyl compounds-derived AGEs.

Subsequently, GO, MGO and BU, three major α-carbonyl compounds which can be generated from both glycoxidation and lipid oxidation, were selected as glycation reagents to prepare AGEs-rich glycated proteins. β-Casein (β-CN) and β-lactoglobulin (β-Lg) which differ in their structures, numbers of glycation sites (Lys and Arg residues) and native digestibility (Pinto et al., 2014; Takagi, Teshima, Okunuki, & Sawada, 2003), were selected as the substrates for glycation and *in vitro* digestion assays. Digestion was conducted in a two-step *in vitro* gastrointestinal digestion system. In addition to analyzing the degree of hydrolysis (DH), we also evaluated the digested peptides, to elucidate the nutritional significance and underlying mechanism of digestibility changes in glycated proteins.

2. Materials and methods

2.1. Materials

Bovine β-CN (≥ 98% purity identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)), β-Lg (> 90% purity identified by SDS-PAGE), GO (40% aqueous solution), MGO (40% aqueous solution), BU (analytical standard) and all enzymes used for the simulated gastrointestinal digestion, including pepsin and pancreatin, were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Glycation model

Glycation was performed in phosphate buffer (50 mM, pH 7) containing 5 mg/mL of protein and 1 or 10 mM GO, MGO or BU. These mixtures were heated in a water bath at 95 ± 1 °C for 1 h in sealed glass vials (10 mL). β-CN and β-Lg were heated independently as control samples. After incubation, the sample was each dialyzed at 4 °C for 1 day using dialysis tubes (3-kDa molecular weight cut-off) to ensure the removal of excess dicarbonyls and salts. The dialyzed solutions were lyophilized and stored at − 20 °C prior to the digestion assay.

2.3. In vitro digestion

An *in vitro* static digestion system was established according to Minekus et al. (2014). The activity of pepsin and pancreatin was each evaluated according to the procedure in the supplementary information of this document. A lyophilized protein (20 mg) was redissolved in 4 mL of simulated gastric fluid (SGF, containing 13.8 mM HCl, 1.8 mM KH_2PO_4, 50 mM $NaHCO_3$, 100 mM NaCl, 0.2 mM $MgCl_2$, 1 mM $(NH_4)_2CO_3$ and 31.2 mM HCl, pH 3.0). Then, 0.1 mL of pepsin (9 mg/mL) was added to obtain a final activity of 500 units/mL. Gastric

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