



Heating and glycation of β -lactoglobulin and β -casein: Aggregation and in vitro digestion



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ABSTRACT

Heat treatment is commonly used in dairy technology. Usually, milk is heated and cooled many times before to obtain the final product and milk components are then subject to cumulative heat treatments. In the present work, we investigated the heat induced aggregation (90 °C until 24 h) of two model proteins, β -lactoglobulin (β -Lg) and β -casein (β -CN), in the presence of glucose and subsequent consequences on simulated gastro-duodenal digestion. Protein aggregation and digestion were monitored using polyacrylamide gel electrophoresis, size exclusion chromatography, dynamic light scattering and transmission electron microscopy. Concomitant heating and protein glycation affect aggregation kinetics as well as protein sensitivity to enzymatic digestion. Spherical covalently linked aggregates were favored in the case of β -CN in the presence of glucose. Glucose limited the formation of twisted fibrils from β -Lg. We clearly showed that aggregates of both proteins formed in the presence of glucose were more resistant to enzymatic digestion. Those formed from β -Lg being highly resistant and still present at the end of simulated gastro-duodenal process. These findings underline the importance not only of the aggregation as such but also of the nature of formed aggregates on protein digestibility.

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1. Introduction

Cow milk contains approximately 3.3% of proteins, 80% of which are caseins that precipitated by acidification of milk at pH 4.6 against 20% soluble whey proteins (Fox & McSweeney, 2003, chap. 1; Walstra, Wouters, & Geurts, 2006, chap. 1). In bovine milk β -casein (β -CN) is the major casein among the four identified casein molecules (α_{s1} -, α_{s2} -, β - and κ -casein) and β -lactoglobulin (β -Lg) is the major protein in whey (Farrell et al., 2004).

β -CN and β -Lg are important not only for their nutritional value but also for their functional properties. β -CN is a flexible molecule that exhibits a lot of segmental motion, little regular secondary structure and is close to a random structure. Above a critical concentration (CMC), it has high propensity to self-assemble into micellar nearly spherical particles (Andrews et al., 1979; O'Connell, Grinberg, & de Kruijff, 2003). β -CN does not contain cysteine residue in its primary sequence (Farrell et al., 2004). In contrast to β -CN, β -Lg has a globular structure, composed of nine anti-parallel β -sheets and one α -helix, and contains two intramolecular disulphide bonds and one free potentially reactive sulphydryl group (Kontopidis, Holt, & Sawyer, 2004). The use of these proteins as ingredient in the food industry requests a better knowledge of their

behavior in solution as well as of interactions with other food components, in particular during processing.

Heat treatment being one of the most widely used unit operation in the food industry. Before obtaining final product, the raw material can undergo cumulative thermal treatments, which modify the properties and behavior of components in the final product. Aggregation and chemical modifications of proteins throughout interaction with other molecules are among the heat-promoted reactions (Li, Enomoto, Ohki, Ohtomo, & Aoki, 2005; Liu & Zhong, 2013). Aggregation kinetics, type and size of aggregates are of paramount importance on the texture of dairy matrices and consequently their functional and nutritional qualities, e.g. texture, digestibility and bioavailability of nutriment (Nicolai, Britten, & Schmitt, 2011; Parada & Aguilera, 2007). The occurrence of a chemical reaction, for example the well-known Maillard reaction between reducing sugar and protein amino-groups, can completely modify the abovementioned aggregation process. Some studies already reported on the effect of reducing sugars on the behavior and heat-induced characteristics of milk proteins, β -Lg or β -CN after treatments under different times/temperatures to assess either protein solubility (Bouhallab, Morgan, Henry, Mollé, & Léonil, 1999), modified sites on protein molecules (Lima, Moloney, & Ames, 2009), digestibility and immunoreactivity (Corzo-Martínez, Soria, Belloque, Villamiel, & Moreno, 2010), structural modifications (Corzo-Martínez, Moreno, Olano, & Villamiel, 2008) and/or covalent crosslinking pathways (Le, Holland, Bhandari, Alewood, & Deeth, 2013; Pellegrino, van Boekel, Gruppen,

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Resmini, & Pagani, 1999). In a previous study, we showed that glucose modifies the aggregation kinetic of β -Lg at neutral pH and consequently the morphology of formed aggregates (Pinto et al., 2012).

In the present study, we conducted a comparative study to determine, under similar experimental treatments, the effects of glucose on the heat-induced aggregation of β -CN, a random coil protein and β -Lg, a globular protein and the relative digestibility of formed aggregates.

2. Materials and methods

2.1. Materials

Fresh bovine raw milk was obtained from the experimental dairy farm (INRA, Rennes, France). The method described by Fox and Guiney (1972) was used to prepare β -CN (a mixture of A1 and A2 variants) that was further purified by ion-exchange chromatography according to a modified method of Guillou, Miranda, and Pelissier (1987). β -Lg variant A was prepared from the milk of homozygous cows as reported by Léonil et al. (1997), in which membrane processes and low temperatures (below 56 °C) were used. The final purity of freeze-dried proteins was 95% and 98% for β -CN and β -Lg respectively, as determined by reversed-phase HPLC analysis. Protein concentration was determined by measuring the absorbance at 280 nm, using extinction coefficient values of 0.458 and 0.937 g/L/cm for β -CN and β -Lg, respectively. All the chemicals and standards used were obtained from Sigma Aldrich (Saint-Quentin-Fallavier, France).

2.2. Glycation experiments

Glycation was performed with glucose in aqueous system. Glucose–protein mixtures were prepared in sodium phosphate buffer 100 mM, pH 7, containing 0.3 mM of β -Lg, 0.4 mM of β -CN and 37.5 mM glucose corresponding to molar excess of glucose, i.e., 8 moles of glucose per mole of protein amino-groups ($-\text{NH}_2$). Control samples were prepared as well without adding glucose. Model system solutions were heated at 90 °C for 24 h in 2 mL test tubes, that were removed in predetermined times between 0 and 24 h, and immediately cooled in an ice-water bath. Each treatment was performed at least in duplicate.

2.3. In vitro digestion of protein aggregates

Simulated gastro-intestinal digestions were performed according to the protocol of Dupont, Mandalari, Molle, Jardin, Léonil, et al. (2010) with modifications. Digestions were performed in two successive steps with duodenal enzymes always added on samples which had already been digested with pepsin. Porcine gastric mucosa pepsin (activity 3800 U/mg of protein) was added to give 182 U of pepsin/mg of β -Lg or β -CN (0.1 mM, final concentration). Aliquots (0.1 mL) were removed over the 60 min digestion time course. Pepsinolysis was stopped by raising the pH using 500 mM sodium bicarbonate. Before duodenal proteolysis, the pH of samples was adjusted to 6.5 by addition of 100 mM NaOH and the following components (duodenal digestion) were added to reach the indicated final concentrations: 4 mM sodium taurocholate, 4 mM sodium glycodeoxycholate, 26.1 mM Bis–Tris buffer, pH 6.5, bovine α -chymotrypsin (activity 52.6 U/mg of protein) to give 0.4 U/mg of β -Lg or β -CN, porcine trypsin (activity 13.4 U/mg of protein) to give 34.5 U/mg of β -Lg or β -CN. Aliquots (0.1 mL) were removed over the 30 min digestion time, and proteolysis stopped by addition of a twofold excess of soybean Bowman–Birk trypsin–chymotrypsin inhibitor above that calculated to inhibit trypsin and chymotrypsin in the digestion mix.

2.4. Sample analyses

2.4.1. Lithium dodecyl sulfate–PAGE analysis

The samples were analyzed by polyacrylamide gel electrophoresis (PAGE) using NuPAGE® Novex® (4–12%, Bis–Tris Mini Gels 1.5 mm,

Invitrogen™). Samples containing either 12 μ g of undigested proteins or 40 μ g digested proteins were mixed with NuPAGE® lithium dodecyl sulfate (LDS) sample buffer, heated at 95 °C for 10 min, cooled to room temperature, and electrophoretically resolved on 4–12% NuPAGE Bis–Tris gels (Invitrogen, Carlsbad, CA, USA). Gels were stained with Coomassie Brilliant Blue R250. A high molecular weight marker kit (3–200 kDa, Mark 12™ Unstained Standard, Invitrogen™) was used for gel calibration.

2.4.2. Size exclusion chromatography (SEC)

The separation was achieved by SEC using a BioSep–SEC–S4000 column, size 300 $\times$ 7.8 mm (Phenomenex, Le Pecq, France), connected to a Waters chromatography system. Elution of samples (25 μ L) was achieved with 50 mM sodium phosphate buffer, pH 7, at 0.5 mL/min for 30 min, and detection of eluting proteins was performed at 214 nm. The standard proteins used for calibration were apoferritin (481.2 kDa); BSA (66 kDa); and α -lactalbumin (14.2 kDa).

2.4.3. Advanced glycation end product (AGE) fluorescence

Advanced glycation end products were determined according to the protocol described by Birlouez-Aragon et al. (1998) except that experiments were carried out in 96-microwell black plate format. Fluorescence measurements were performed with 0.2 mL samples on a Spectra Max M2 fluorescence spectrophotometer (Molecular Devices, Sunnyvale, California). Excitation and emission wavelengths were 330 and 420 nm, respectively. When necessary, samples were diluted in pH 7 phosphate buffer.

2.4.4. Transmission electron microscopy (TEM)

Drops of 0.005 mM protein suspensions (β -Lg or β -CN) were deposited onto glow-discharged carbon-coated microscopy grids. The liquid in excess was blotted with filter paper and a drop of distilled water was deposited on the preparation in order to rinse out the residual glucose and buffer salts. The water in excess was blotted and, prior to drying, the preparations were negatively stained with 2% (w/v) uranyl acetate. The samples were observed using a JEOL JEM 1400 microscope operating at 120 kV. Images were recorded on camera Gatan Orius SC 1000 at Microscopy Imaging Center platform (MRic), situated in Rennes, France.

2.4.5. Dynamic light scattering

Particle size analysis was made on a Zetasizer nano ZS (Malvern Instruments, Orsay, France). Dynamic light scattering at a fixed angle of 173° was performed using a He–Ne laser, with $\lambda = 633$ nm. Samples were equilibrated at 20 °C for 2 min and the characteristics of water was considered as solvent to the samples, the refractive index of water was 1.330, and the viscosity of water was 1.0031 mPa s at 20 °C. This information was used in the computerization of the data to obtain the relaxation time distribution, which was transformed into a distribution of hydrodynamic diameters, D_h , using Stokes–Einstein relationship: $D = kT / 3\pi\eta D_h$, where k is Boltzmann's constant, T is the absolute temperature and η is the viscosity of water at 20 °C.

3. Results and discussion

3.1. Heat-induced aggregation and glycation of β -Lg and β -CN

The heat-induced aggregation of β -Lg and β -CN was studied at pH 7 either in the absence or presence of eight fold molar excess of glucose over the free protein amino-groups. As we already performed for β -lactoglobulin under the same experimental conditions (Pinto et al., 2012), the glycation of β -CN was monitored by fluorescence intensity of advanced end product (AGE) according to Birlouez-Aragon et al. (1998). Fig. 1 shows the evolution of AGE after heating of β -CN at 90 °C over 24 h with and without glucose. As expected AGE formation increased with increasing heating time. The final fluorescence values

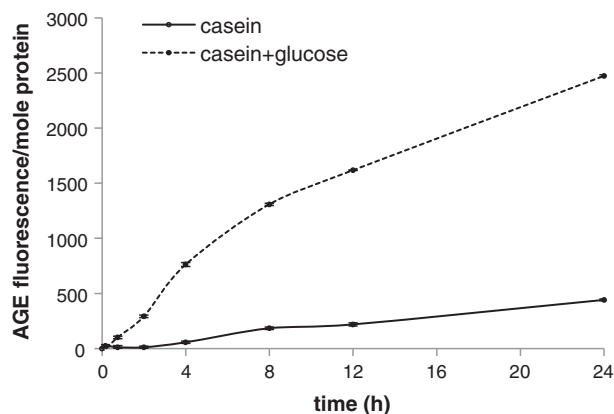


Fig. 1. Formation of fluorescent advanced glycation end products as a function of heating time of β -casein solution (0.4 mM) at 90 °C and pH 7.0, without (dashed line) and with 37.5 mM glucose (solid line). AGE was expressed per mole of protein.

reached after the 24 h heating is close to that already found for a mixture β -Lg and glucose (Pinto et al., 2012). However, the formation of AGE from β -CN was faster than from β -LG. This is rather expected since β -CN is a flexible unstructured protein with more accessible reactive sites.

Heat-induced aggregation and glycation of β -Lg and β -CN were monitored by LDS–PAGE technique (Fig. 2). Compared with a control without glucose under the studied time interval, the presence of glucose

slows down the kinetic of β -Lg aggregation, as was already reported by our group (Pinto et al., 2012). This feature can be observed by the slower onset aggregates at the stacking gel for the glycated β -Lg samples. In contrast, for β -CN, aggregated species appeared faster when heating was performed in the presence of glucose. This protein associates mainly by hydrophobic bonds, which were dissociated in LDS–PAGE, but also through covalent bonds as already described by Pellegrino et al. (1999). The covalently linked large aggregates of β -CN in the presence of glucose that accumulated in the stacking gel were detected after prolonged heating time (> 12 h). The occurrence of large aggregates in the presence of glucose was confirmed by size exclusion chromatography that showed progressive increase of protein material eluted at the void volume during the heating time, as compared to samples without glucose (Fig. 3). It should be noted that high molecular weight material was already found in native unheated β -CN (Fig. 3A & B). Before heating, β -CN exists in micellar form since the concentration used was above the critical micellar concentration (CMC) of β -CN (Kull, Nylander, Tiberg, & Wahlgren, 1997). To form these micelles, β -CN monomers associate into micelles via hydrophobic bonding (O'Connell et al., 2003). Using small-angle neutron scattering Ossowski et al. (2012) modeled the structure of β -CN as a polydisperse ellipsoid core in solution. The protein micelles with an average hydrodynamic diameter of 28 nm (see below) were completely dissociated during LDS–PAGE analysis. After heating, hydrophobic interactions are reinforced, concomitantly to the occurrence of covalent bonds between proteins inside the micelles. These heat-induced covalently aggregated micelles were not dissociated in the LDS–PAGE (Fig. 2B). According to the pioneer work by Andrews et al. (1979), the thermally-induced β -CN aggregates are spherical with a radius of about 100 Å, the interior of which is relatively disordered, and the β -CN molecules remain in a largely flexible, hydrated conformation. The presence of glucose during heating promoted the formation of large covalently linked aggregates throughout covalent complexation of pre-formed β -CN small aggregates or micelles (Fig. 2B). Lysine residues and to a lesser extent arginine residues are

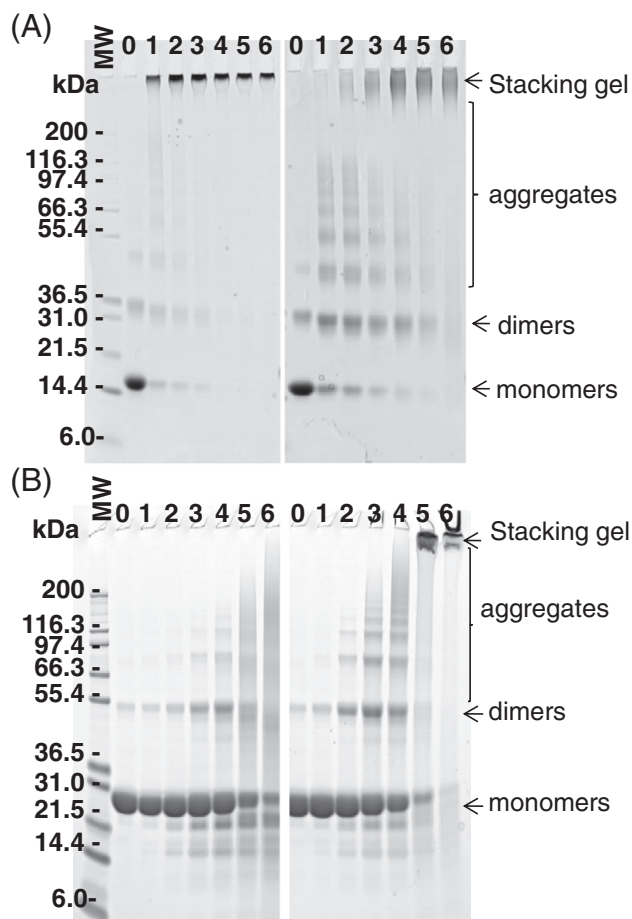


Fig. 2. LDS–PAGE of heated (90 °C) β -lactoglobulin (A) and β -casein (B) without (left) or with (right) added glucose. (0) native and heated protein at different times: (1) 10 min, (2) 45 min, (3) 2 h, (4) 4 h, (5) 12 h and (6) 24 h. MW = molecular weight marker.

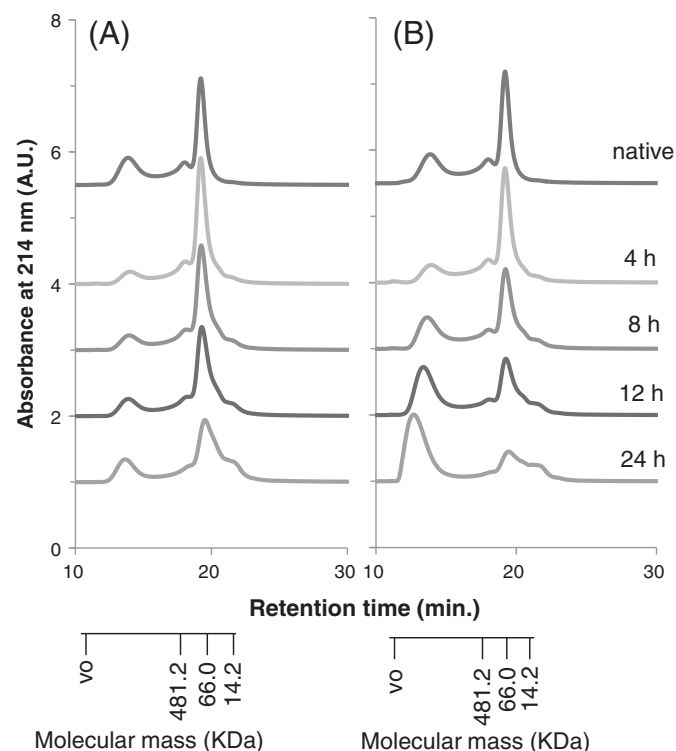


Fig. 3. Size exclusion chromatography profiles of β -casein (0.4 mM) heated at 90 °C during various times without (A) and with (B) glucose (37.5 mM). The secondary x-axis indicates the elution times of molecular weight markers; vo = void volume.

known to be the sites of heat induced glycation of β -CN (Lima et al., 2009) as well as of β -Lg (Mollé, Morgan, Bouhallab, & Léonil, 1998). Several studies tried to identify the nature of covalent bonds and possible pathways in protein crosslinking. Pellegrino et al. (1999) studied the covalent crosslinking of heated β -CN in the presence and absence of Glucose. They found that crosslinking via dehydroalanine formation predominated in the absence of glucose while the formation of pentosidine, an advanced Maillard reaction product, consecutively to Lys-Arg crosslink prevailed in the presence of glucose. Similar results were obtained in reconstituted milk samples since heating in the presence of lactose favored crosslink via Maillard reaction products that inhibit the formation dehydroalanine bonds through competition for lysine residues (Al-Saadi, Easa, & Deeth, 2013). Le et al. (2013) showed that methylglyoxal, a Maillard reaction product, was a major cause of protein aggregation and crosslinking in the presence of lactose but the chemical nature of cross-links was not characterized.

Furthermore, the formation of covalent oligomers, not dissociated in LDS-PAGE, was also detected in non-glycated β -CN samples after heating (Fig. 2B). These oligomers entered the separation gel, indicating that they are smaller than those formed in the presence of glucose. Nevertheless, these oligomers were not too small to be separated by SEC as they were eluted in the void volume (Fig. 3A). Also, the SEC profiles of heated β -CN exhibited a peak eluted after the monomer (Fig. 3). The corresponding material could be attributed to β -CN derived peptides as it is well-known that heat-induced proteolysis of caseins occurs following incubation at high temperature (Morales & Jiménez-Pérez, 1998).

The morphology of heat-induced aggregates from β -Lg and β -CN was observed by transmission electron microscopy (TEM). β -CN showed mainly small spherical particles without fibrils and some clusters of these spheres (Fig. 4). The presence of these clustered species is probably due to the effect of negative staining, i.e., uranyl ions that can bind to proteins. Self-association of β -CN into small aggregates without formation of fibrils was recently reported by Ossowski et al. (2012) using scattering techniques and cryo-TEM. TEM images show also the differences between glycated and non-glycated β -CN (Fig. 4B

and D). Spherical particles of about 10–20 nm were found for aggregates from β -CN heated 90 °C for 24 h without glucose. Spherical shape of particles was maintained in the presence of glucose but with greater diameter (20–50 nm). However, this glucose effect on particle size after heating of β -CN was not detected by dynamic light scattering measurements (Table 1). β -CN aggregates exhibited average hydrodynamic diameters of about 30–35 nm after the 24 h heating independent of the presence of glucose. Also, heating does not affect significantly the size of β -CN as this protein already exists in micellar form in solution at concentration higher than CMC as discussed above. The hydrodynamic diameters from dynamic light scattering measurements are slightly larger than those extracted from TEM images, probably due to protein dehydration during sample preparation for TEM experiments. The morphology of heat-induced aggregates of β -CN differs from that observed for treated β -Lg (Fig. 4A). As we have already shown (Pinto et al., 2012), in contrast to what happens with β -CN, glucose slows down the kinetic of fibril formation from β -Lg. Separated or aggregated twisted and long fibrils (up to 900 nm) were observed when β -Lg was heated without glucose. In the presence of glucose, a mixture of aggregates and shorter fibrils of about 50 to 200 nm in length was observed. The width of the fibrils of about 10 nm was not modified by the presence of glucose. Dynamic light scattering data (Table 1) showed an increase of β -Lg aggregate size after 2 h heating from around 7 to 63 nm and 79 nm, with and without glucose, respectively. The same trend was observed after 24 h, confirming our previous results on the fact that glucose retarded the kinetic of β -Lg fibrillation (Pinto et al., 2012).

3.2. In vitro digestion of heated/glycated proteins

Native and heated proteins (90 °C for 24 h) with or without glucose were subjected to in vitro digestion. The LDS-PAGE profile of the native proteins digested by gastric and duodenal enzymes were presented in Fig. 5. Native β -Lg was not significantly digested at the end of simulated gastro-duodenal digestion cycle. These results are in accordance with those reported by other authors even if the percent of hydrolyzed protein by duodenal enzymes varied from one study to another (Barbé

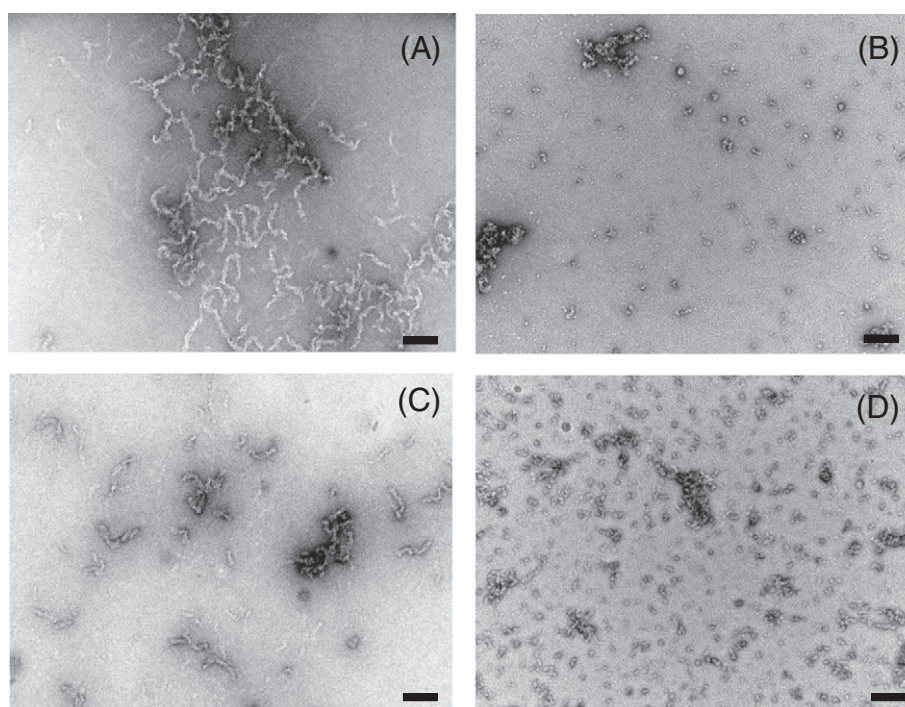


Fig. 4. Transmission electron microscopy micrographs of β -lactoglobulin heated at 90 °C for 4 h (left) and β -casein heated for 24 h (right) without (A and B) and with (C and D) added glucose. Bars = 100 nm.

Table 1

Hydrodynamic size (nm) of the native and 90 °C for 2 and 24 h heated β -casein and β -lactoglobulin with or without glucose.

Heating time (h)	Size (nm)		β -Casein	β -Casein + glucose
	β -Lactoglobulin	β -Lactoglobulin + glucose		
0	7.4 $\pm$ 0.5 ^a	7.4 $\pm$ 0.5 ^a	27.7 $\pm$ 1.0 ^b	27.7 $\pm$ 1.0 ^b
2	77.8 $\pm$ 1.8 ^c	58.1 $\pm$ 5.6 ^d	30.2 $\pm$ 1.8 ^e	29.7 $\pm$ 2.1 ^{b,e}
24	86.1 $\pm$ 4.4 ^f	62.2 $\pm$ 6.3 ^d	34.9 $\pm$ 0.3 ^g	31.7 $\pm$ 1.0 ^e

Values are mean of two independent samples measured 3 times each $\pm$ SD. Values with the same letter were not significantly different (ANOVA, $p < 0.05$).

et al., 2013; Dupont, Mandalari, Mollé, Jardin, Duboz, et al., 2010; Mandalari, Mackie, Rigby, Wickham, & Mills, 2009). In contrast, native β -CN was highly sensitive to digestion as it disappeared completely after a 20 min incubation with pepsin. The behavior of these two proteins toward digestion is related to their structures, β -Lg is a well-structured globular protein, less accessible to enzymatic attacks while β -CN is known to be highly sensitive as an intrinsically unstructured protein.

The digestion profiles of both proteins changed after heating 24 h at 90 °C (Figs. 6 and 7). Heated β -CN appeared to be slightly more sensitive to pepsin compared to un-heated protein since 5 min incubation with pepsin was enough to digest formed β -CN aggregates (Fig. 6). Heated β -Lg was also completely digested after a 40 min incubation

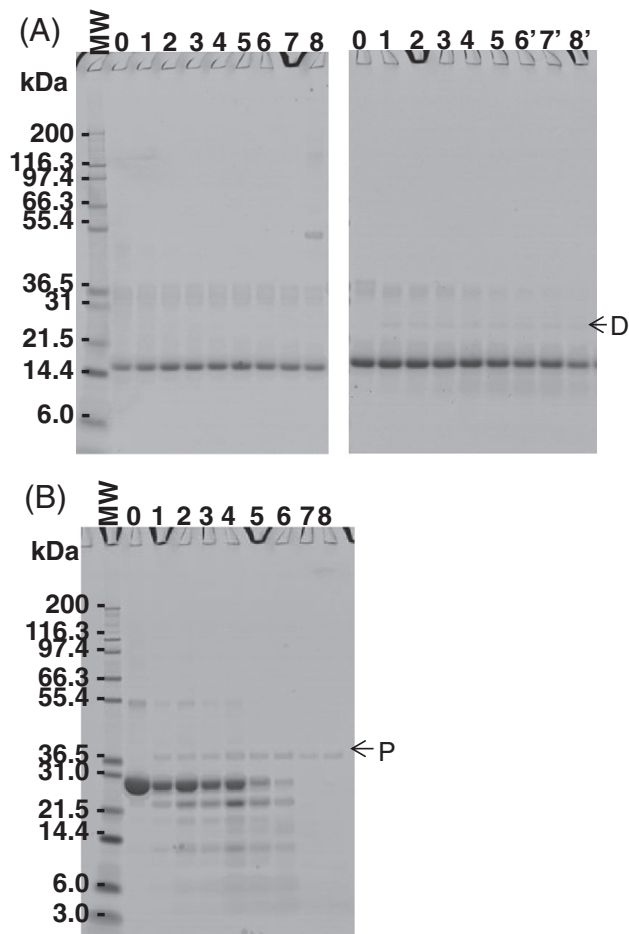


Fig. 5. LDS–PAGE profiles of native (A) β -lactoglobulin and (B) β -casein digested by gastric (left) and duodenal (right) enzymes as a function of digestion time: without enzyme (0), or with enzyme for (1) 0 min, (2) 1 min, (3) 2 min, (4) 5 min, (5) 10 min, (6') 15 min, (6 and 7') 20 min, (8') 30 min, (7) 40 min, and (8) 60 min. MW = molecular weight marker. Arrows indicate pepsin (P) and duodenal enzymes (D).

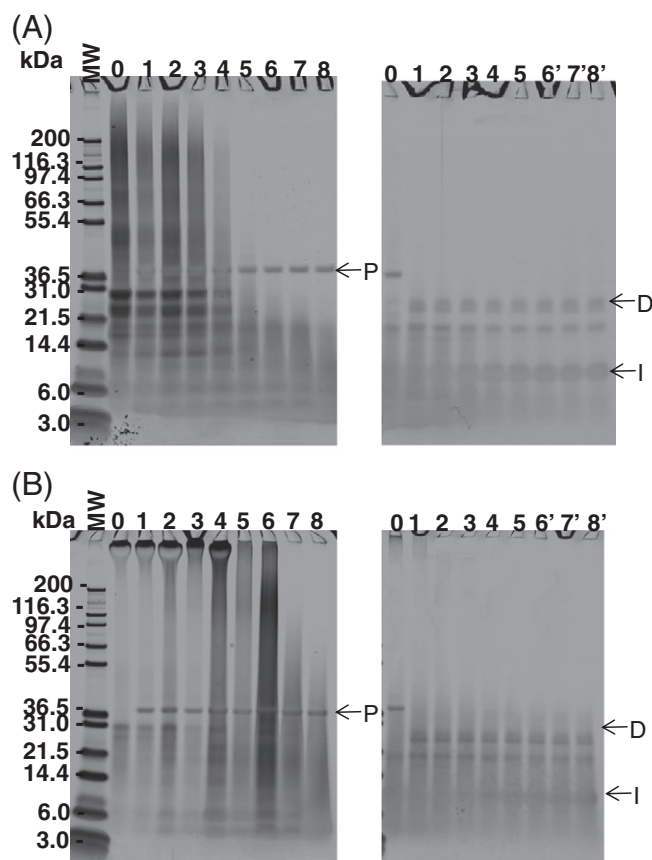


Fig. 6. LDS–PAGE profiles of digestion kinetics of β -casein heated at 90 °C for 24 h without (A) or with (B) glucose. Digestion by gastric (left) and duodenal (right) enzymes: without enzyme (0), or with enzyme for (1) 0 min, (2) 1 min, (3) 2 min, (4) 5 min, (5) 10 min, (6') 15 min, (6 and 7') 20 min, (8') 30 min, (7) 40 min, and (8) 60 min. MW = molecular weight marker. Arrows indicate pepsin (P), duodenal enzymes (D) and inhibitor of duodenal enzymes (I).

under gastric conditions (Fig. 7). These results confirm those already reported in *in vitro* studies which showed that heat treatments render β -Lg susceptible to cleavage by pepsin and duodenal enzymes, i.e. trypsin and chymotrypsin (Barbé et al., 2013; Kim et al., 2007; Mandalari et al., 2009). The presence of sugar during heating limited the heat induced sensitivity of these proteins to enzymatic attacks as shown by the LDS–PAGE analyses (Figs. 6 and 7). Aggregated forms of β -CN were still detected after 60 min incubation with pepsin. Interestingly, LDS–PAGE shows the progressive digestion of large aggregates (Fig. 6B, lane 2) to small ones that enter the gel (Fig. 6B, lane 6). Nearly, complete digestion to peptides was achieved before adding duodenal enzymes, as it was the case without glucose during heating. Glucose induced more drastic change in the case of β -Lg. As shown in Fig. 7A, the 40 min incubation under gastric conditions was sufficient for total digestion of aggregates from β -Lg heated without glucose. In contrast, aggregates formed in the presence of glucose (top of the gel) are still present at the end of gastro-intestinal digestion process (Fig. 7B). Observation of digested samples by TEM supports the resistance of glycated β -Lg to enzymatic digestion (Fig. 8). Ecroyd et al. (2008) showed that fibrillar structures from κ -casein were much more resistant to degradation by trypsin than native protein. This trend was not observed in the case of heat-induced twisted fibrils of β -Lg. TEM image of digested β -Lg shows that these fibrils were broken down into small particles. Digestion of sample heated in the presence of glucose showed a different picture with a co-presence of small and large particles. These results confirm those of Corzo-Martínez et al. (2010) who showed that glycation with galactose or tagatose impaired β -Lg proteolysis and immunoreactivity. They suggest that protein aggregation

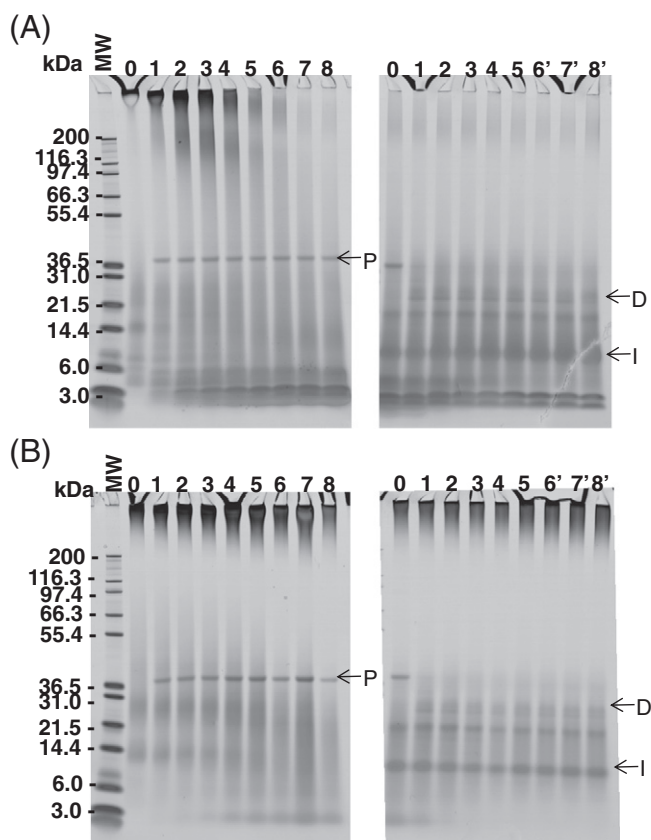


Fig. 7. LDS-PAGE profiles of digestion kinetics of β -lactoglobulin heated 90 °C for 24 h without (A) and with (B) glucose. Digestion by gastric (left) and duodenal (right) enzymes: without enzyme (0), or with enzyme for (1) 0 min, (2) 1 min, (3) 2 min, (4) 5 min, (5) 10 min, (6') 15 min, (6 and 7') 20 min, (8') 30 min, (7) 40 min, and (8) 60 min. MW = molecular weight marker. Arrows indicate pepsin (P), duodenal enzymes (D) and inhibitor of duodenal enzymes (I).

induced in the presence of sugars may protect the protein during *in vitro* proteolysis. Also, the covalent crosslinks between protein molecules induced via Maillard products could strengthen the resistance of peptidic bonds to digestion. In contrast, glycation has been shown to increase protein proteolysis due to conformational changes produced during glycation (Morgan et al., 1999; Yeboah et al., 2004). This apparent discrepancy may be attributed to the progress of Maillard reaction in various studies, early versus very advanced, that control the aggregation state of the proteins.

4. Conclusion

The presence of glucose highly impacts the heat-induced aggregation behavior of β -Lg, a well-structured globular protein and of β -CN, an unstructured protein. Heating leads to twisted fibrils in the case of β -Lg against spherical particles in the case of β -CN. Structural studies are needed to determine the exact chemical nature of crosslinking involved in the two types of aggregates. Glucose retarded the aggregation kinetic of β -Lg and favored the formation of large covalently linked aggregates of β -CN. Aggregates produced in the presence of glucose, especially from β -Lg were shown to be more resistant to simulated gastrointestinal digestion. Even if severe heating was applied in the present study (90 °C, 24 h), the occurrence of such type of resistant aggregates in formulated food is not excluded. The biological properties and consequences deserve to be evaluated in a future work.

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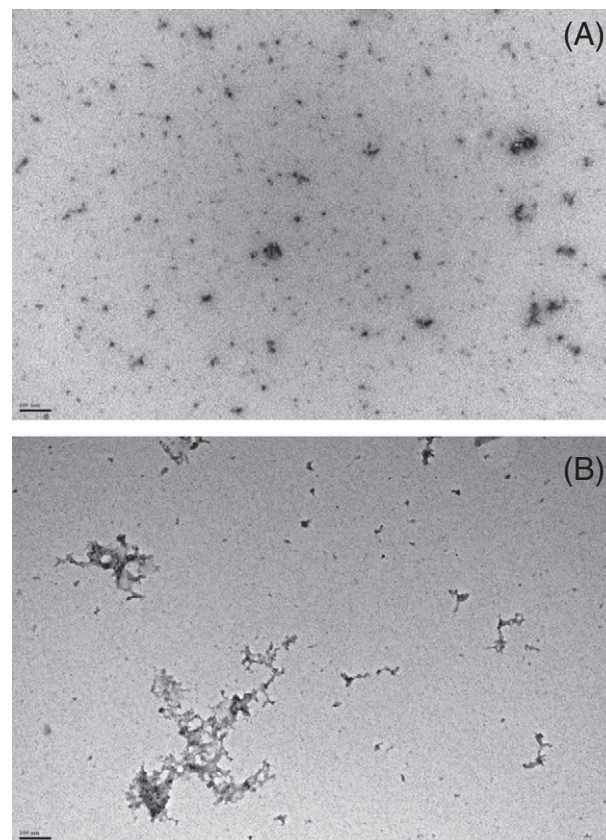


Fig. 8. TEM images of heated β -lactoglobulin (90 °C for 4 h) without (A) and with (B) glucose and subsequently subjected to gastric/duodenal digestion. Bars = 100 nm.

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