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Effect of milk protein glycation and gastrointestinal digestion on the growth of bifidobacteria and lactic acid bacteria

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ABSTRACT

In this paper, β-lactoglobulin (β-Lg) and sodium caseinate (SC) have been glycated via Maillard reaction with galactose and lactose and, subsequently, the effect of glycoconjugates hydrolyzed under simulated gastrointestinal digestion on the growth of pure culture of *Lactobacillus*, *Streptococcus* and *Bifidobacterium* has been investigated. Glycopeptides were added to the growth media as the sole carbon source. None of the bacterial strains was able to grow in hydrolysates of native and control heated β-Lg and SC. However, glycopeptides were fermented, in different degree, by *Lactobacillus* and *Bifidobacterium* and hardly any effect was detected on the growth of *Streptococcus*. Digested β-Lg glycoconjugates showed a strain-dependent effect whereas growth profiles of bacteria when hydrolysates of SC glycoconjugates were used as substrates were very similar, regardless of the strain. A general preference towards peptides from β-Lg/SC glycated with galactose, particularly at the state of the reaction in which the highest content in the Amadori compound tagatosyl-lysine is present, was observed. SC glycoconjugates were quickly fermented by some strains, promoting their growth in a greater extent than β-Lg complexes or even glucose. Therefore, from the results obtained in this work it can be concluded that conjugation of both milk proteins with galactose and lactose via the Maillard reaction could be an efficient method to obtain novel food ingredients with a potential prebiotic character.

1. Introduction

Nowadays, it is possible to dispose of products which afford not only the basic nutritional value but also a wide range of benefits that can contribute to improve consumer well-being. In this respect, due to the fact that severe health disorders can be related to the gastrointestinal function, some of the functional ingredients more demanded by consumers are those with a positive effect on gut microbiota (Saarela et al., 2002). These ingredients are non-digestible carbohydrates with well-known properties, being lactulose (Tuohy et al., 2002), tagatose (Laerke and Jensen, 1999; Laerke et al., 2000; Jensen et al., 2001), fructooligosaccharides (FOS) and galacto-oligosaccharides (GOS) (MacFarlane et al., 2008) some of the main oligosaccharides considered as prebiotics.

On the other hand, deliberated glycosylation via the Maillard reaction (MR) or glycation has been largely investigated during the last years as a promising approach to alter the functional properties of proteins for food purposes (Oliver et al., 2006a). Concretely, it has been demonstrated that milk proteins (caseins and whey proteins), usually obtained as by-products in the dairy industry, can increase their degree of applicability by means of glycation. Thus, controlling the reaction conditions it is possible to obtain neoglycoconjugates with different glycation and aggregation degrees, being preferred, in general, the early steps of the reaction (Jimenez-Castaño et al., 2005a, 2005b; Kato, 2002; Oliver et al., 2006a). Particularly interesting could be the case of the conjugation of milk proteins with galactose and lactose, which are isomerised to tagatose and lactulose, respectively, giving rise to the corresponding Amadori compounds, tagatosyl- and lactulosyl-lysine. Taking into account the prebiotic properties of tagatose and lactulose, it could be expectable some effect of these glycoconjugates on gut microbiota.

In spite of the number of works on protein glycation, any information about intestinal absorption and endogenous metabolisation of Amadori compounds is still fragmentary. According to several authors, it seems that these initial products of the MR are scarcely digested and excreted, being fermented in the distal colon (Erbersdobler and Faist, 2001; Finot, 1973; Finot, 2005; Lee and Erbersdobler, 1994; Sanz et al., 2007). In this sense, the study of the effect of the Amadori compounds tagatosyl- and lactulosyl-lysine could be of great interest, since such compounds might reach more distal areas of gut than tagatose and lactulose and be fermented by the present microbiota. This is particularly relevant since the incidence of certain chronic gut disorders is much higher in the descending colon and rectum than in the first sections of the large gut (Gibson et al., 2004). Mills et al. (2008), using *in vitro* assays with faecal

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slurries, pointed out that glycated protein bovine serum albumin modulated the colonic microbiota of volunteers with ulcerative colitis towards a more detrimental composition with significant increases in putatively harmful bacteria and decreases in dominant and putatively beneficial bacterial group. This trend was not completely confirmed in non-ulcerative colitis volunteers.

Thus, the purpose of this work has been to study the effect of glycoconjugates obtained by glycosylation via the Maillard reaction of milk proteins (β-lactoglobulin and sodium caseinate) with galactose and lactose, and subsequently subjected to simulated gastrointestinal digestion, on the growth of twelve potential probiotic strains of *Lactobacillus*, *Streptococcus* and *Bifidobacterium* to broaden the knowledge on the role of Amadori compounds in the modulation of the human gut microbiota.

2. Materials and methods

2.1. Materials

Glucose (Glu), galactose (Gal), lactose (Lac), tagatose (Tag), lactulose (Lu) and bovine β-lactoglobulin (β-Lg) (mixture of A and B variants) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and sodium caseinate (SC) (Rovita FN 5) was obtained from Proveedora Hispano Holandesa, S.A. (Barcelona, Spain).

Streptococcus salivarius ZL50-7, *Lactobacillus reuteri* R13, *Lactobacillus plantarum* CLC17, *Lactobacillus delbrueckii* ZL96-27, *Lactobacillus brevis* CLC23, *Lactobacillus gasseri* Lc9 and *Bifidobacterium breve* 26 M2 belonged to the culture collection of the Nutrition, Bromatology and Food Technology Department of the Faculty of Veterinary Science (Universidad Complutense de Madrid). *Streptococcus thermophilus* STY-31, *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB12 and *Lactobacillus casei* LC-01 strains had been previously purified from a commercial symbiotic product (Simbiotic Drink; Priégola, Madrid, Spain) at our laboratory (Tabasco et al., 2007). *Lactobacillus plantarum* IFPL722, isolated from cheese, was taken from our IFPL culture collection. All cultures were maintained at −80 °C in MRS broth (Pronadisa, Madrid, Spain) or, in the case of *S. salivarius* ZL50 and *S. thermophilus* STY-31, in M17 broth (Pronadisa), both supplemented with glycerol (40% v/v) and subcultured in MRS or M17 broth before use in experiments.

2.2. Preparation and purification of glycoconjugates

Carbohydrates, Gal or Lac, and β-Lg in a weight ratio of 1:1 or 2:1, respectively, were dissolved in 0.1 M sodium phosphate buffer, pH 7.0 (Merck, Darmstadt, Germany), and lyophilized. The β-Lg-Gal powders were kept at 40 and 50 °C for 24 and 48 h, respectively (Corzo-Martinez et al., 2008), while the β-Lg-Lac powders were kept at 60 °C for 8 and 48 h (Table 1) (Fenaille et al., 2004), under vacuum in a desiccator equilibrated at an a_w of 0.44, achieved with a saturated K_2CO_3 solution (Merck). In addition, control experiments were performed with β-Lg stored at 40, 50 and 60 °C without reducing sugars during the same periods (control heated β-Lg). Incubations were performed in duplicate, and all analytical determinations were performed at least in duplicate.

Carbohydrates, Gal or Lac, and sodium caseinate (SC) in a weight ratio of 0.2:1 were dissolved in 0.1 M sodium phosphate buffer, pH 7.0 (Merck) and lyophilized (Corzo-Martinez et al., 2010b). The SC-Gal powders were kept at 60 °C and 50 °C for 4 and 72 h, respectively, while the SC-Lac powders were kept at 60 °C for 8 and 24 h (Table 1), under vacuum in a desiccator equilibrated at an a_w of 0.67 (Oliver et al., 2006b), achieved with a saturated solution of $CuCl_2$ (Sigma-Aldrich). In addition, control experiments were performed with SC stored at 50 and 60 °C without reducing sugars during the same periods (control heated SC). Incubations were performed in duplicate, and all analytical determinations were performed at least in duplicate.

After incubation, the products were reconstituted in distilled water to a protein concentration of 1 mg/mL. To remove free carbohydrate, 2 mL portions were ultrafiltered through hydrophilic 3 kDa cut-off membranes (Centricon YM-3, Millipore Corp., Bedford, MA) by centrifugation at 1548 *g* for 2 h. After removal of free Gal or Lac, samples were reconstituted in distilled water at a concentration of 2 mg/mL for further analysis (Corzo-Martinez et al., 2008).

Taking into account the analyses of structural characterization previously carried out in our laboratory (Corzo-Martinez et al., 2008, 2010a; Laparra et al., 2011), two types of glycoconjugates were obtained at different stages of the Maillard reaction after incubation of every combination of carbohydrate and protein under conditions indicated above (Table 1). One of them, in early stages of the MR (β-Lg:Gal [24 h, 40 °C], β-Lg:Lac [8 h, 60 °C], SC:Gal [4 h, 60 °C] and SC:Lac [8 h, 60 °C]), consisted primarily of complexes with a high content of the Amadori compound tagatosyl- or lactulosyl-lysine and a low aggregation level, while those glycoconjugates incubated under more severe conditions, in advanced stages of the MR (β-Lg:Gal [48 h, 50 °C], β-Lg:Lac [48 h, 60 °C], SC:Gal [72 h, 50 °C] and SC:Lac [24 h, 60 °C]), exhibited a high glycation degree, considerable amounts of advanced glycation products (AGEs and melanoidins), and a high aggregation level.

2.3. In vitro gastrointestinal digestion

All SC and β-Lg glycoconjugates, as well as the control heated SC/β-Lg samples were digested *in vitro* by following the simplified procedure described by Moreno et al. (2005). This digestion model was based on *in vivo* data obtained by gastric and duodenal aspiration and from collection of effluent from ileostomy volunteers at the Institute of Food Research (Norwich, UK).

For the gastric digestion step, glycoconjugates (3 mg) were dissolved in 1 mL of simulated gastric fluid (SGF, 0.15 M NaCl, pH 2.5). The pH was adjusted to 2.5 with 1 M HCl if necessary. A solution of 0.32% (w:v) porcine pepsin (EC 3.4.23.1) in SGF (pH 2.5) (Sigma, activity of 3300 U/mg of protein) was added at an approximately physiological ratio of enzyme to substrate (1:20, w:w). The digestion was performed at 37 °C for 2 h. For the intestinal digestion step, the pH was increased to 7.5 with 40 mM NH_4CO_3 (Panreac, Barcelona, Spain) dropwise to inactivate pepsin, and the following was added to adjust the pH to 6.5 and simulate a duodenal environment: (i) a

Table 1
Structural features of glycoconjugates used for the bacterial growth study.

Glycoconjugates	Incubation conditions	Structural features	Reference
β-Lg:Gal	40 °C, 24 h	Maximum content in the Amadori compound tagatosyl-lysine and non-aggregated	Corzo-Martinez et al. (2008)
	50 °C, 48 h	Highly glycated and aggregated	Corzo-Martinez et al. (2008)
β-Lg:Lac	60 °C, 8 h	Maximum content in the Amadori compound lactulosyl-lysine and non-aggregated	Laparra et al. (2011)
	60 °C, 48 h	Highly glycated and aggregated	Laparra et al. (2011)
SC:Gal	60 °C, 4 h	Maximum content in the Amadori compound tagatosyl-lysine and non-aggregated	Corzo-Martinez et al. (2010a, 2010b)
	50 °C, 72 h	Highly glycated and aggregated	Corzo-Martinez et al. (2010a, 2010b)
SC:Lac	60 °C, 8 h	Maximum content in the Amadori compound lactulosyl-lysine and non-aggregated	Corzo-Martinez et al. (2010a, 2010b)
	60 °C, 24 h	Highly glycated and aggregated	Corzo-Martinez et al. (2010a, 2010b)