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Polysaccharides reduce *in vitro* IgG/IgE-binding of β-lactoglobulin after hydrolysis

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Abstract

We investigate the influence of charged polysaccharides such as gum arabic and low methylated pectin (LMP), and of un-charged polysaccharides such as xylan, on the *in vitro* digestibility of β-lactoglobulin (β-lg) and on the *in vitro* IgG- and IgE-binding of its digestion products. β-Ig was hydrolyzed by pepsin, and then by a trypsin/chymotrypsin mixture, in dialysis bags. SDS–PAGE electrophoresis was used to determine protein hydrolysis and immunoblotting in the presence of cow's milk, and rabbit or human antibodies were used to assess the *in vitro* IgG- and IgE-binding of the digestion products. The results showed that polysaccharides influenced protein digestibility. The IgG- and IgE-bound hydrolysis products were dependent on the enzymatic hydrolysis and on the presence of polysaccharides. In all cases, IgG-binding was lower in the presence of any of the polysaccharides, and IgE-binding was non-existent or considerably reduced with LMP and xylan. In this study, LMP and xylan were the polysaccharides that most effectively reduced the immuno-reactivity of the hydrolysis products. The eventual reduction of *in vivo* allergenicity of milk-based food products has to be confirmed.

Keywords: β-Lactoglobulin; Enzymatic hydrolysis; Dialysis bag; Protein–polysaccharide interactions; IgG/IgE-binding

1. Introduction

Cow's milk and dairy products are major nutrients in the human diet, especially during infancy. However, cow's milk is an allergenic product. In most studies, cow's milk protein allergy is most common in young infants, with an incidence of 2–6% (Hill & Hosking, 1996, 1997; Hosking, Heine, & Hill, 2000). Although all cow's milk proteins appear to be potential allergens, even if present only in trace amounts, the main allergens seem be found in the casein fraction, in the whey-protein fractions, such as β-lactoglobulin (β-lg) and in α-lactalbumin (α-la) and albumin (Besler, Steinhart, & Paschke, 2001; Wal, 1998).

The various processing methods used in formulating cow's milk-based foods may affect their allergenicity. The molecular basis of changes in allergenic activity is the inactivation or destruction of epitope structures or increased accessibility to cryptic epitopes when the native allergen

Abbreviations: β-lg, β-lactoglobulin; α-la, α-lactalbumin; LMP, low methylated pectin; MW, molecular weight; MWCO, molecular weight cut-off; T/C, trypsin/chymotrypsin.

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is denatured (Besler et al., 2001). The allergenicity of cow's milk is due to some fragments of proteins containing epitopes, not hydrolyzed by digestive enzymes, and able to cross the intestinal barrier to trigger allergic responses (Jaffuel, Demoly, & Bousquet, 2001; Schmidt, Meijer, Slangen, & van Beresteijn, 1995).

β-lg, the most abundant fraction of cow's milk whey proteins, is considered to be a dominant allergen (Sélo et al., 1999). At physiological pH, β-lg presents a dimeric structure. The monomer is an 18-kDa globular protein of 162 amino acids with four disulphide bonds per dimer and one free cysteine group. Intramolecular bonds maintain the structural integrity of protein and assure its stability against hydrolysis and heat (Hambling, Mc Alpine, & Sawyer, 1992). The β-lg three-dimensional structure consists of nine strands of anti-parallel β-sheets, eight of which form a hydrophobic barrel on one side with an α-helix (Kontopidis, Holt, & Sawyer, 2004). Asselin, Amiot, Gauthier, Mourad, and Herbert (1988) and Asselin, Herbert, and Amiot (1989) suggested that selective proteolysis of whey milk by pepsin and chymotrypsin was the most efficient enzymatic combination for reducing the allergenicity due to β-lg. Nakamura, Sado, Syukunobe, and Hirota (1993), using proteases of plant or bacterial origin, demonstrated that extensive protein hydrolysis produced a significant reduction in the antigenicity of whey proteins, and suggested that the antigenicity of proteins is decreased by destruction of epitopes present on the native protein.

On the other hand, some dairy products, such as yoghurt drinks, flavoured milk, custards, ice creams and puddings, contain soluble polysaccharides of plant origin, such as alginates, carrageenans, pectins, gum arabic, guar gum or locust bean gum. Interactions between these polysaccharides and milk proteins improve the texture and stability of dairy products (Dickinson, 1998; Syrbe, Bauer, & Klostermeyer, 1998). These interactions could reduce the digestion of proteins; a higher digestibility lowers the allergenicity. Some authors have shown that β-lg allergenicity is indeed modified in the presence of mono- or polysaccharides (Hattori, Numamoto, Kobayashi, & Takahashi, 1997, 2000; Morgan et al., 1998). Lactosylated β-lg, produced by the Maillard reaction during heating, changes the epitopic area (Morgan et al., 1998). Conjugates of β-lg and anionic acidic saccharides, such as carboxymethyl dextran and alginic acid, have enhanced some functional properties such as heat stability, emulsifying capacity, and reduced immunogenicity (Hattori et al., 1997, Hattori, Numamoto, Kobayashi, & Takahashi, 2000) due to changes in protein conformation. Conversely, Hattori et al. (2000) have shown, in another study, that conjugated products like β-lg with cationic saccharides, such as glucosamine, chitopentoase, increased the antigenicity and immunogenicity of β-lg, whereas chitosan reduced these.

The main objective of the present study was to determine the effects of gum arabic and low methylated pectin (LMP), anionic polysaccharides widely used in the food industry, and a neutral oligomer, xylan, extracted from *Palmaria palmata*, red seaweed, authorized for human consumption, on the immuno-reactivity of the hydrolysis products of β-lg. Some experiments have demonstrated the inhibition of digestive enzymes by protein digestion products (Gonzalez-Tello, Camacho, Jurado, Paez, & Guadex, 1994; Hsu, Vavak, Satterlee, & Miller, 1977; Weber & Nielsen, 1991), so, in this study, β-lg was hydrolyzed by a two-step method, according to Savoie and Gauthier (1986), using dialysis bags for the second step (intestinal digestion). Previous work has revealed a difference of N released after β-lg trypsin/chymotrypsin (T/C) according to the molecular weight cut-off (MWCO) of dialysis bag used (Mouécoucou et al., 2003).

2. Materials and methods

2.1. Materials

Acid-processed bovine β-lg isolate (batch 838) was kindly provided by Lactalis (Retiers, France). This was not a purified preparation, but it will be referred to as β-lg. It contained (g/100 g): 89.8% protein (N × 6.38) with 90% of β-lg. Powdered gum arabic (batch 97 J 716; 90.0% polysaccharide, including 15% glucuronic acid and 58% neutral sugar) was a gift from Colloides Naturels International (CNI, Rouen, France). Xylan (batch PP28-4-00; 80.4% pentose, 13.2% hexose) was extracted from a Rodophyceae, *P. palmata* or dulse, by the Laboratoire de Pathologie Cellulaire et Moléculaire en Nutrition (Faculté de Médecine Vandoeuvre-lès-Nancy, France). Low methylated pectin (LMP) (batch 0B800; 87% polysaccharide, degree of esterification: 37–41%) was provided by Degussa Texturant Systems (Boulogne-Billancourt, France).

Porcine pepsin (activity 3800 U/mg protein, EC 3.4.23.1), bovine trypsin (13,800 U/mg, EC 3.4.21.4), porcine chymotrypsin (51 U/mg, EC 3.4.4.5) and thimerosal, were purchased from Sigma (France). The BioRad broad molecular range marker, was used as a standard marker (BioRad, France).

2.2. IgG and IgE sera

Rabbit antiserum raised against cow's milk, obtained after incubating for 63 days, and rabbit pre-immune serum were used (Agrobio, France). Serum from six patients with known cow's milk sensitivity was pooled and used for our experiments. The cow's milk-specific IgE level determined by RAST was >100 kU/l, as measured by the Pharmacia CAP system. Normal serum, from patients without a cow's milk hypersensitivity reaction, was used as an IgE negative control.

2.3. Methods

2.3.1. Preparation of β-lg powder/polysaccharide mixtures

Powdered β-lg, containing 40 mg of nitrogen (1.7% w/v of protein), was dissolved in 15 ml of 0.17 M sodium

phosphate (pH 7) buffer. Polysaccharides were dissolved in the same buffer at the same concentration (1.7% w/v). The LMP dispersion solution was obtained at heat temperature (70 °C during 1 h). The dispersions were left overnight at 4 °C to allow complete hydration of the macromolecules. The stock β-lg and polysaccharide dispersions were blended so as to obtain a 0 (control β-lg) and 50% (w/w) solution; 50 mg/l of thimerosal was added to all solutions.

2.3.2. Enzymatic hydrolysis

2.3.2.1. Peptic digestion. One milliliter of pepsin in 0.02 N HCl (1 mg/ml) was added to 15 ml of β-lg/polysaccharide mixtures (E/S: 1/250). To simulate *in vivo* gastric digestion, the pH of the dispersions was progressively reduced from 7 to 2 over 2 h by adding 0.02 N HCl with a peristaltic pump (Ecoline, Ismatec, Zurich, Switzerland) (flow rate: 80 μl/min) at 37 °C. The digestion was stopped when the pH reached 2 by cooling the samples and freeze-drying.

2.3.2.2. T/C digestion in dialysis bags. The *in vitro* digestion of β-lg/polysaccharide mixtures was carried out at 37 °C in a dialysis cell (Serna, Laval, Quebec, Canada) using the two-step hydrolysis method developed by Savoie and Gauthier (1986), with minor modifications of the first step. In this step, peptic digestion of the β-lg/polysaccharide mixtures was done as described in the previous section. Peptic digestion was stopped by raising the pH to 8 with 2 N NaOH. In the second step, the samples were transferred to dialysis bags with MWCOs 1000 or 8000 Da (SpectraPor 6, Interchim, Montluçon, France). Then, 1 ml of a T/C mixture (1/2.3, w/w) at a weight concentration of 2.5 mg/ml (E/S: 1/50) was then added. Digestion was done during 6 h. The experiments were done in triplicate. Retentates were freeze-dried and kept until analyzed.

2.3.3. Protein assay

The protein content of all the samples was estimated using the bicinchoninic acid assay (Commercial kit, Interchim, France).

2.3.4. Electrophoresis and immunoblotting

Polysaccharides, β-lg after peptic hydrolysis, and the retentates were characterized by sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), according to the method of Laemmli and Favre (1973). Using 0.1% (w/v) SDS and 5% (v/v) 2-mercaptoethanol, SDS–PAGE was performed with 4.5% polyacrylamide concentration gel in 0.125 M Tris (pH 6.8) and with a 15% polyacrylamide separation gel in 0.38 M Tris (pH 8.8). Proteins were stained by Coomassie blue (R 250 0.1%) (w/v) in 50% ethanol (v/v) and 10% acetic acid (v/v). A prestained, broad-range protein marker was run with each gel to estimate the MW of the proteins.

The *in vitro* IgG/IgE bindings of undigested β-lg and its hydrolysis products were analyzed by immunoblotting, using the sera containing anti-cow's milk IgG from rabbits or IgE from patients. Samples were loaded onto a gel for SDS–PAGE as described above. After electrophoresis, the proteins were transferred onto polyvinyl difluor membranes (Millipore, France) by applying a constant current of 300 mA for 60 min with a semi-dry blotting (Trans-Blot, Bio-Rad, France). Cut strips (4 mm) were used for immunodetection. All the patient or rabbit sera were diluted with Tris buffer saline (TBS, 50 mM, pH 7.5) containing 0.05% (v/v) Tween 20 (TBS-Tween). The total incubation volume was 2 ml per strip. The strips were washed three times for 15 min with 0.1% TBS-Tween between successive incubation steps. They were incubated with primary antibodies (rabbit anti-cow's milk or rabbit pre-immune, 1:10,000; allergenic or non-allergenic patient sera 1:10) for 12 h at 4 °C. Biotinylated anti-rabbit IgG or biotinylated anti-human IgE (Biovaley, France) were subsequently incubated (1:2000) for 2 h at room temperature. Antigen-antibody conjugates were visualized by their reaction to streptavidin alkaline phosphatase (1:1000) (Biovaley, France) for 30 min. Enzymatic staining was performed with 4-nitroblue tetrazolium chloride and 5bromo-4chloro- 3-indolylphosphate, using a commercial kit (Sigma, France).

3. Results

3.1. SDS–PAGE electrophoresis patterns

3.1.1. Electrophoresis of β-lg after peptic hydrolysis (Fig. 1)

Fig. 1 shows the SDS–PAGE electrophoresis pattern of undigested β-lg and β-lg after peptic digestion, with or without polysaccharides. The MW of the bands was estimated by comparison with the MW of the standard and with purified milk proteins (data not shown) and by a gel scanning programme (VersaDoc, Biorad, France). Bands with MW values of 40, 30, 18 and 14 kDa, corresponding to β-lg dimer, β-casein, β-lg monomer and α-la, respectively, were clearly visible in the undigested β-lg (lane 6). The β-lg dimer, α-la and β-lg, were poorly digested by pepsin. A pale band with MW 23 kDa appeared in lane 2 (β-lg control). β-Casein (MW: 30 kDa) was considerably reduced by peptic hydrolysis. The presence of gum arabic (lane 3), LMP (lane 4) or xylan (lane 5) did not affect the peptic digestibility of the proteins.

3.1.2. Electrophoresis of β-lg after peptic and T/C hydrolysis (Fig. 2)

In order to investigate the efficiency of enzymatic degradation, a second step of β-lg digestion was performed with the mixture of pancreatic enzymes (T/C mixture) at pH 7, in dialysis bags with different MWCO.

Fig. 2 shows the electrophoresis pattern of undigested β-lg and retentates in the presence or absence of polysaccharides in dialysis bags. All native proteins were hydrolyzed, and bands corresponding to the peptides or residual proteins were observed in the electrophoresis pattern of the retentates of both dialysis bags. The β-lg hydrolyzed without polysaccharides in the dialysis bag with a MWCO of 1000 Da (lane 2) showed pale and intense bands with

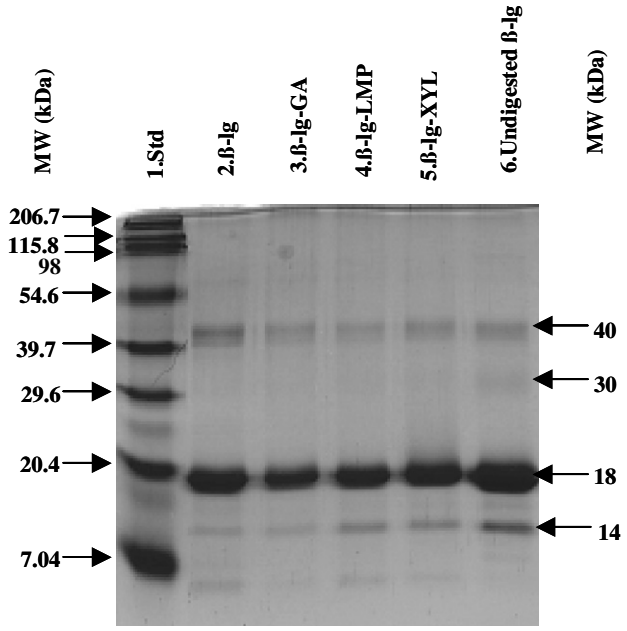

Fig. 1. SDS–PAGE electrophoresis of β-lg after peptic hydrolysis. Lane 1: Std (standard, marker of MW); lane 2: β-lg control (without polysaccharide); lane 3: β-lg-gum arabic (GA); lane 4: β-lg-low methylated pectin (LMP); lane 5: β-lg-xylan (XYL); lane 6: undigested β-lg. Each lane was loaded with 10 μg of proteins.

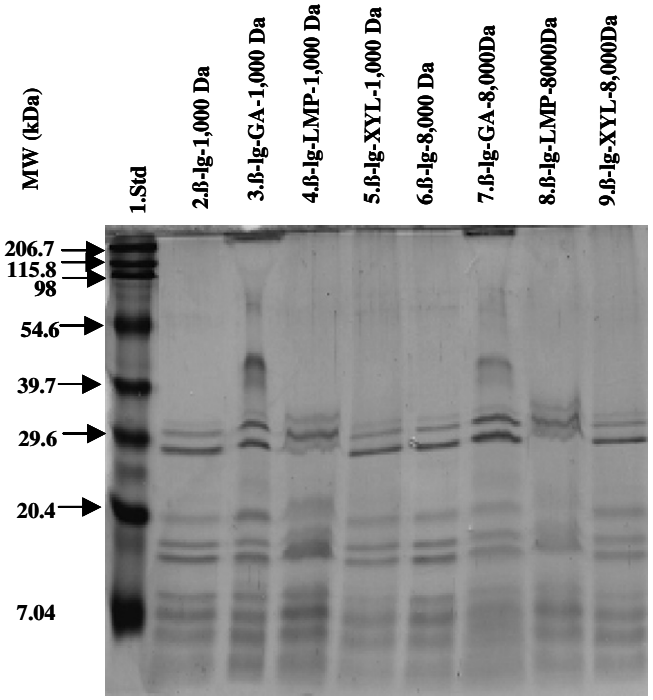

Fig. 2. SDS–PAGE electrophoresis of retentates after β-lg peptic, followed by T/C digestion in dialysis bags. β-lg T/C digestion in dialysis bag with MWCO of 1000 Da; lane 2: β-lg without polysaccharide; lane 3: β-lg-gum arabic (GA); lane 4: β-lg-low methylated pectin (LMP); lane 5: β-lg -xylan (XYL). β-lg T/C digestion in dialysis bag with MWCO of 8000 Da; lane 6: β-lg without polysaccharide; lane 7: β-lg-gum arabic (GA); lane 8: β-lg-low methylated pectin (LMP); lane 9: β-lg (XYL). Lane 1: Std (standard, marker of MW). Each lane was loaded with 150 μg of proteins.

apparent MW values of 30, 29 and 27 kDa. These were followed by three bands with apparent MWs of 18, 17 and 15 kDa, respectively. Four bands, with apparent MWs of 9, 7 and <7 kDa, respectively, were also observed. Adding gum arabic to the β-lg solution (lane 3) modified the electrophoresis profile, with the appearance of two additional bands with apparent MWs of 40 and 23 kDa. The presence of LMP slightly modified the protein hydrolysis electrophoresis pattern (lane 4); the band with apparent MW of 18 was slightly reduced, bands with apparent MW 15 and 7 kDa were more intense, with a slight reduction of the band with apparent MW 9 kDa. The electrophoresis pattern of the retentate containing xylan (lane 5) was comparable to that of the β-lg control. The same electrophoresis profile was observed when the hydrolysis was performed without polysaccharides in the dialysis bag, with a MWCO of 8000 Da, (lane 6). In this dialysis bag, gum arabic (lane 7), LMP (lane 8) and xylan (lane 9) had the same effect as in the dialysis bag with a MWCO of 1000 Da, but bands with MW < 27 kDa were slightly less intense.

3.1.3. Electrophoresis of polysaccharides

No bands appeared on SDS–PAGE electrophoresis of all polysaccharides (results not shown).

3.2. Immunoblots

3.2.1. IgG immunoblots

The immunoblot of undigested β-lg shows that all the proteins were recognized by cow's milk rabbit antibodies (Fig. 3). The β-lg monomer (MW 18 kDa) had the greatest IgG-binding intensity. On the other hand, the band with a MW of 30 kDa (β-casein), that was reduced after peptic hydrolysis, was recognized by IgG only when hydrolysis was carried out in the presence of polysaccharides (lanes 3–5). When this sample was incubated with a pre-immune rabbit serum, it did not show IgG-binding to cow's milk proteins (results not shown).

The immunoblot of the retentates reveals several peptides, resulting from the hydrolysis of native proteins of β-lg, recognized by the cow's milk rabbit antibodies (Fig. 4). The most important effect was with the bands with apparent MWs of 29, 18 and 9 kDa. Bands with MW < 7 kDa were not recognized by IgG. Gum arabic (lane 3) and xylan (lane 5) did not modify the IgG-binding peptides. Conversely, in lane 4, IgG-binding was reduced; only the bands with MW values of 29 and 7 kDa displayed IgG-binding. The same results were also observed with the retentates of the dialysis bags with an MWCO 8000 Da (lanes 6–9).

3.2.2. IgE immunoblots

Proteins of undigested β-lg were bound to human IgE antibodies (Fig. 5, lane 6). The band with an apparent MW of 14 kDa, corresponding to α-la, displayed slight IgE-binding. The incubation of the same sample with the serum of patients not sensitized to cow's milk proteins did not bind to IgE (results not shown).

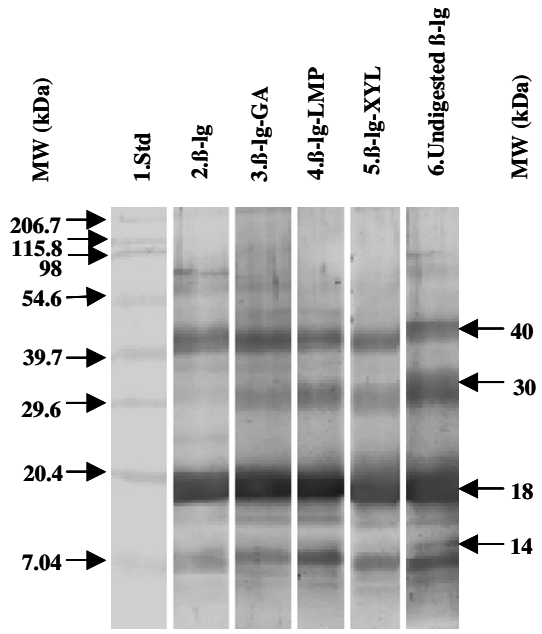

Fig. 3. Immunoblot of IgG-binding proteins after the peptic hydrolysis ofβ-lg. Lane 1: Std (standard, marker of MW); lane 2: β-lg without polysaccharide; lane 3: β-lg-gum arabic (GA); lane 4: β-lg-low methylated pectin (LMP); lane 5: β-lg-xylan (XYL); lane 6: undigested β-lg. Each lane was loaded with 10 μg of proteins.

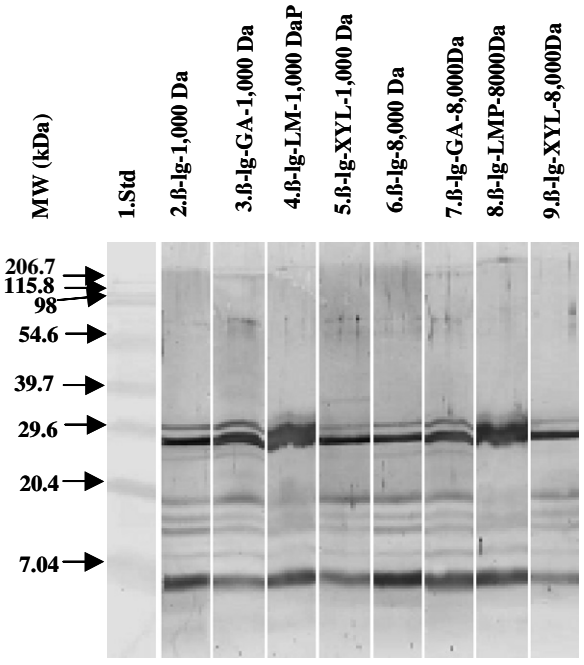

Fig. 4. Immunoblot of IgG-binding proteins of retentates after β-lg peptic digestion, followed by T/C digestion in dialysis bags. β-lg T/C digestion in dialysis bag with an MWCO of 1000 Da; lane 2: β-lg without polysaccharide; lane 3: β-lg-gum arabic (GA); lane 4: β-lg-low methylated pectin (LMP); lane 5: β-lg -xylan (XYL). β-lg T/C digestion in dialysis bag with an MWCO of 8000 Da; lane 6: β-lg without polysaccharide; lane 7: β-lg-gum arabic (GA); lane 8: β-lg-low methylated pectin (LMP); lane 9: β-lg (XYL). Lane 1: Std (standard, marker of MW). Each lane was loaded with 150 μg of proteins.

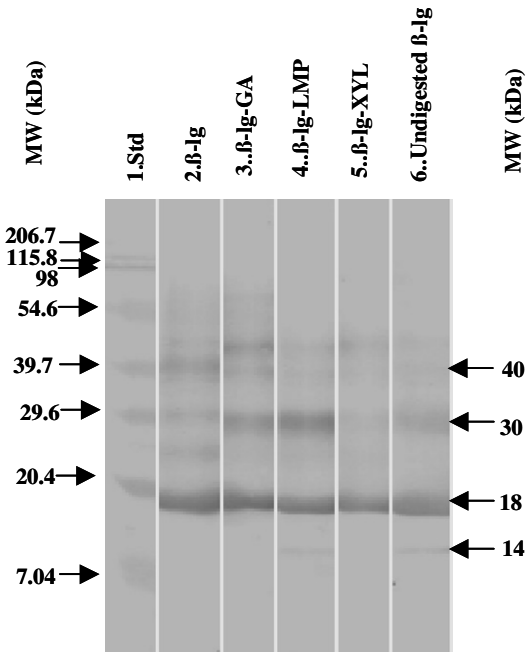

Fig. 5. Immunoblot of IgE-binding proteins after peptic hydrolysis of β-lg. Lane 1: Std (standard, marker of MW); lane 2: β-lg without polysaccharide; lane 3: β-lg-gum arabic (GA), lane 4: β-lg-low methylated pectin (LMP), lane 5: β-lg-xylan (XYL), lane 6: undigested β-lg. Each lane was loaded with 10 μg of proteins.

After the peptic hydrolysis of β-lg, all the bands observed in the electrophoresis pattern bound to the IgE antibodies (Fig. 5). Human antibodies recognized the band with an MW of 30 kDa, particularly in the presence of gum arabic (lane 3) or LMP (lane 4). IgE-binding of α-la was observed only when hydrolysis was performed in presence of LMP. In all cases, the greatest IgE-binding was observed for the band with an apparent MW of 18 kDa, corresponding to the β-lg monomer. After T/C hydrolysis, conversely to their binding to IgG, the peptides of the retentates displayed little affinity for IgE (Fig. 6). Only two bands (29 and 18 kDa) showed only slightly binding to IgE. This was greater for the control β-lg (lane 2) and in the presence of gum arabic (lane 3), in both dialysis bags. In the presence of LMP (lanes 4 and 8) or xylan (lanes 5 and 9), IgE-binding was either absent or much less intense than in the previous case.

4. Discussion

In this study, we compared the effects of polysaccharides on the *in vitro* peptic hydrolysis and peptic followed by T/C hydrolysis of the β-lg in dialysis bags and on the *in vitro* IgG and IgE-binding of the hydrolysis products. The *in vitro* digestion method used simulates the *in vivo* situation by using a two-step hydrolysis (Gauthier, Vachon, & Savoie, 1986; Savoie & Gauthier, 1986). This makes it possible to monitor the immuno-reactivity change of the proteins and of their hydrolysis products during the different steps of digestion. Pepsin did not hydrolyze the α-la and the β-lg, as was reported by some authors, particularly in the case of β-lg (Mahé et al., 1996; Mullaly, Mehra, & Fitzgerald,

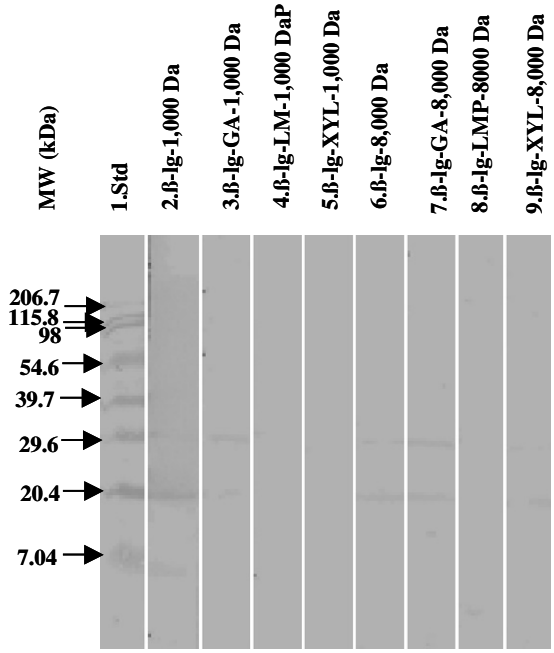

Fig. 6. Immunoblot of IgE-binding proteins of retentates after peptic digestion of β-lg, followed by T/C digestion in dialysis bags. T/C digestion of β-lg in dialysis bag with an MWCO of 1000 Da; lane 2: β-lg without polysaccharide; lane 3: β-lg-gum arabic (GA); lane 4: β-lg-low methylated pectin (LMP); lane 5: β-lg-xylan (XYL). β-lg T/C digestion in dialysis bag with an MWCO of 8000 Da; lane 6: β-lg without polysaccharide; lane 7: β-lg-gum arabic (GA); lane 8:β-lg-low methylated pectin (LMP); lane 9: β-lg-xylan (XYL). Lane 1: Std (standard, marker of MW). Each lane was loaded with 150 μg of proteins.

1998; Reddy, Kella, & Kinsella, 1988). Pepsin is a predigestive enzyme that cleaves the bonds between aromatic amino acids (tyrosine and phenylalanine) and facilitates hydrolysis by pancreatic enzymes (Mahé et al., 1996; Reddy et al., 1988; Savoie & Gauthier, 1986).

β-lg, hydrolyzed by the combination of digestive enzymes (P, T/C), produced several peptides. These findings are consistent with those obtained in a previous study, where the digestibility of the same β-lg sample, estimated by the nitrogen release through a dialysis bag with a MWCO of 1000 Da, was reduced in the presence of all the polysaccharides used (Mouécoucou et al., 2003). The SDS–PAGE pattern of retentates suggests that each polysaccharide has a different effect on the reduction of protein digestibility. Gum arabic reduced the hydrolysis of β-lg sample by the partial retention of some high MW peptides. LMP reduced the hydrolysis of proteins with lower MW (15 and 7 kDa); xylan did not reduce the β-lg breakdown. Gum arabic also has hydrophobic properties (in contrast to pectin and xylan) and is frequently used by the food industry as an aroma-stabilizing emulsifier. It could interact with some specific peptides.

The accessibility of trypsin and chymotrypsin to their site of cleavage could be modified by these polysaccharides. However, in our experiment, their enzymatic activities were not reduced by any polysaccharides (data not shown). Previous studies in our laboratory showed that, at low polysaccharide concentrations, the viscosity of the mixture did not increase (Lamghari El Kossori et al., 2000; Sanchez, Renard, Robert, Schmitt, & Lefebvre, 2002). These observations strongly depend on pH, ionic strength and polysaccharide type. This suggests that polysaccharides interact with digestion products, and particularly with low-MW digestion products, to form complexes that cannot escape from the dialysis bags. These complexes, at pH 8, could be due to ionic interactions between the anionic polysaccharide molecules and positively-charged portions of hydrolysis products, as observed by Imeson, Ledward, and Mitchell (1977), Tolstoguzov (1986) and Xia and Dubin (1994). The same authors also observed non-ionic interactions, such as hydrogen bonding, during the formation of polysaccharide-protein/peptide complexes; this could be the case here too, particularly in the case of xylan, which is a neutral oligomer.

The reaction of rabbit and human antigens to cow's milk revealed considerable IgG/IgE-binding after the peptic hydrolysis of β-lg; this was reduced after T/C hydrolysis. The use of single enzymes may underestimate digestibility, as it did in the experiment of Astwood, Leach, and Fuchs (1996), who treated purified β-lg with simulated gastric fluid for 60 min and concluded that β-lg was still highly allergenic after peptic digestion. However, *in vivo*, the gastric phase is not the most important for protein digestion (Bernier, Adrian, & Vidon, 1988; Ekmeckcioglu, 2002). Other authors have shown that whey protein antigenicity is reduced by a combination of fungal proteinase and a pepsin/pancreatic extract (Asselin et al., 1988; Ena, van Berestejn, Robben, & Schmidt, 1995; Kananen et al., 2000; Nakamura et al., 1993; Svenning, Brynhindsvold, Molland, Langsrud, & Vegared, 2000). The specificity of the enzymes used influenced the epitope areas, which are responsible for the immunological reactions (Gonella & Walker, 1987). These findings indicate that the antigenic responses of β-lg hydrolysates depend considerably on the enzyme used. As both high- (29–30 kDa) and low-MW (7 kDa) peptides displayed IgG-binding, the molecular mass distributions of the hydrolysates did not reflect the degree of reduction of the antigenicity of the proteins reported by Svenning et al. (2000). On the other hand, as polysaccharides influence protein hydrolysis and consequently, the peptides produced, the antigenicity of the hydrolysis products also depended on whether they were present during both steps of the hydrolysis and on their ability.

The difference in response between IgG- and IgE-binding peptides was due to the different epitopes of these antibodies. The presence of IgE-binding epitopes after T/C hydrolysis in the presence of gum arabic suggests that this polysaccharide preserved the IgE epitope of bands with MW 18 and 29 kDa by reducing the protein hydrolysis. The absence or reduction of IgE-binding with the 18-kDa band, when hydrolysis was performed in the presence of LMP and xylan, could be due to the destruction of IgE epitopes or to their inaccessibility associated with change of conformation. Although the mechanism by which the

polysaccharides reduce the β-lg by-hydrolysis products is unknown at this stage of our research, it is evident that their intrinsic properties (nature and structure), are determinant for the ability of peptides release to be recognized or not by IgE.

Previous studies have demonstrated that specific interactions, such as covalent linkage between the polysaccharides and β-lg produced by the Maillard reaction during heat-processing, modify the allergenicity of proteins (Hattori et al., 1997, 2000, 2004; Morgan et al., 1998). Our experiment demonstrates that, even without heating, non-specific interactions between polysaccharides and the hydrolysis products of cow's milk proteins could reduce the antigenicity of milk proteins.

The *in vitro* model used for the hydrolysis of proteins is only a model of digestion and not of absorption. Protein absorption involves several mechanisms, including intracellular digestion, and the presence of a transporter, that are not included in this experimental model. However, as in the context of increased intestinal permeability, where high MW peptides pass through the intestinal barrier, or in young infants, where the intestinal tract is immature (Gonella & Walker, 1987; Heyman, Grasset, Ducroc, & Desjeux, 1988), it is possible that *in vivo*, peptides with IgE-epitopes could cross this barrier and therefore cause an allergic response. The eventual reduction of *in vivo* allergenicity of milk-based food products containing polysaccharides has to be confirmed.

5. Conclusion

This study demonstrates that the *in vitro* allergenicity of β-lg was reduced after peptic hydrolysis, followed by T/C hydrolysis, indicating that some of the IgE/IgG-epitopes were destroyed by these enzymes. The hydrolysis products inside and outside the dialysis bags had IgE/IgG-binding epitopes that were reduced due to the presence of polysaccharides. In this study, LMP and xylan were the most efficient polysaccharides in reducing the immuno-reactivity of the hydrolysis products. The eventual reduction of *in vivo* allergenicity of milk-based food products has to be confirmed. The continuation of this work will lead to the demonstration of the mechanisms, which could explain the effects of polysaccharides on β-lg digestibility. The determination of peptides that resist digestion could lead to a better understanding of the phenomena observed.

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