Binding Interactions Between alpha-glucans from Lactobacillus reuteri and Milk Proteins Characterised by Surface Plasmon Resonance

Diemer, Silja K.; Svensson, Birte; Babol, Linnea N.; Cockburn, Darrell; Grijpstra, Pieter; Dijkhuizen, Lubbert; Folkenberg, Ditte M.; Garrigues, Christel; Ipsen, Richard H.

Published in:
Food Biophysics

DOI:
10.1007/s11483-012-9260-5

Publication date:
2012

Food Biophysics (2012) 7:220–226
DOI 10.1007/s11483-012-9260-5

ORIGINAL ARTICLE

Binding Interactions Between α-glucans from *Lactobacillus reuteri* and Milk Proteins Characterised by Surface Plasmon Resonance

Silja K. Diemer · Birte Svensson · Linnéa N. Babol · Darrell Cockburn · Pieter Grijpstra · Lubbert Dijkhuizen · Ditte M. Folkenberg · Christel Garrigues · Richard H. Ipsen

Received: 13 September 2011 / Accepted: 17 May 2012 / Published online: 14 June 2012

Abstract Interactions between milk proteins and α-glucans at pH 4.0–5.5 were investigated by use of surface plasmon resonance. The α-glucans were synthesised with glucansucrase enzymes from *Lactobacillus reuteri* strains ATCC-55730, 180, ML1 and 121. Variations in the molecular characteristics of the α-glucans, such as molecular weight, linkage type and degree of branching, influenced the interactions with native and denatured β-lactoglobulin and κ-casein. The highest overall binding levels were reached with α-(1,4) compared to α-(1,3) linked glucans. Glucans with many α-(1,6) linkages demonstrated the highest binding levels to κ-casein, whereas the interaction with native β-lactoglobulin was suppressed by α-(1,6) linkages. Glucans with a higher degree of branching generally displayed lower protein binding levels whereas a higher molecular weight resulted in increased binding to κ-casein. The interactions with κ-casein were not pH dependent, whereas binding to denatured β-lactoglobulin was highest at pH 4.0 and binding to native β-lactoglobulin was optimal at pH 4.5–5.0. This study shows that molecular weight, linkage type and degree of branching of α-glucans highly influence the binding interactions with milk proteins.

Keywords α-glucan · Homopolysaccharides · β-lactoglobulin · κ-casein · *Lactobacillus reuteri* · Surface plasmon resonance

S. K. Diemer · R. H. Ipsen
Department of Food Science, University of Copenhagen,
Rolighedsvej 30,
1958 Frederiksberg C, Denmark

B. Svensson · D. Cockburn
Enzyme and Protein Chemistry, Department of Systems Biology,
Technical University of Denmark,
Søltofts Plads, Building 224,
2800 Lyngby, Denmark

S. K. Diemer (✉) · D. M. Folkenberg · C. Garrigues
Innovation, Chr Hansen A/S,
Bøge Allé 10-12,
2970 Hørsholm, Denmark
e-mail: dkskd@chr-hansen.com

L. N. Babol
Department of Food Science, Uppsala BioCenter,
Swedish University of Agricultural Sciences,
Box 7051, 750 07 Uppsala, Sweden

P. Grijpstra · L. Dijkhuizen
Microbial Physiology, Groningen Biomolecular Sciences
and Biotechnology Institute (GBB), University of Groningen,
Nijenborgh 7,
9747 Groningen, The Netherlands

Introduction

Lactic acid bacteria (LAB) forming exopolysaccharides (EPS) are widely used in the manufacture of yoghurt and fermented milk products as they increase viscosity and creaminess while decreasing syneresis.[1, 2] EPS are classified according to their monosaccharide composition. Heteropolysaccharides (HePS) are composed of repeating units of different monosaccharides, e.g. galactose, glucose, rhamnose, etc., and homopolysaccharides (HoPS) solely contain glucose (α-glucans) or fructose (fructans).[3] HePS are formed by LAB commonly applied in yoghurt production and responsible for the desired milk texture despite their rather modest concentration of up to 2.4 gL^{-1} [4] and often below 0.6 gL^{-1} [5]. By contrast HoPS were reported to be produced in considerably larger amounts up to 40 gL^{-1}.[6] *Leuconostoc* and *Lactobacillus* species form HoPS in the

presence of sucrose.[3] A variety of *Lactobacillus reuteri* strains synthesise fructans and glucans with differing molecular properties, e.g. molecular weight (MW), distribution of linkage types and degree of branching.[7] The linkage types and branching affect the conformational flexibility of the glucans.[8]

Glucans such as dextran are applied as texturising and stabilising agents improving food product structure.[9] The effects of glucans and EPS in foods depend on their molecular characteristics and interactions with proteins.[10,11] When used in fermented milk products, glucans should be able to interact with the milk proteins under the actual process conditions. Milk is intensively heat treated (e.g. at 95 °C for several minutes) prior to the production of yoghurt or other fermented milk products, resulting in denaturation of whey proteins, which become more prone to interact [12] and associate with κ-casein either on the surface of the casein micelle or as soluble complexes.[13] Mainly β-lactoglobulin participate in this complex formation. During yoghurt production and also post-acidification, milk changes from about pH 6.5 to pH 4.1; during this process EPS is produced, which may interact with milk proteins.[1] It may interact with the κ-casein, as well as with partly denatured whey protein, of which β-lactoglobulin constitutes the greatest fraction.[12] The formation of the protein network may initiate at pH 5.2–5.3,[14] and hence EPS-protein interactions occurring at pH 5.5 to 4.0 are important for the texture.

Binding between EPS and milk proteins can be monitored by surface plasmon resonance (SPR) analysis[15] involving immobilisation of the various milk proteins onto a sensor chip and passing EPS in solution over the sensor surface. When EPS binds to the protein a change in the SPR response is detected, which is proportional to the amount of bound EPS. Even weak interactions can be detected over time by this technique.[15,16] SPR has been extensively applied in life science and drug discovery, but has also several applications in food science.[17] In relation to milk, SPR was used to quantify β-casein[18], casein interactions,[19] and recently to describe casein–carrageenan[20] and milk protein–HoPS interactions,[15] respectively.

The interactions of HoPS from three *Lactobacillus* species with immobilised β-casein, β-lactoglobulin and κ-casein were previously monitored by SPR at pH 4.0–5.5[15] and found to be highly dependent on the bacterial strain, pH and the milk protein. In the present study, glucans from different strains of *Lactobacillus reuteri* (ATCC-55730, 180, ML1 and 121) of varying molecular weight, linkage types, and degree of branching[7,21–24] were analysed for binding to native β-lactoglobulin, denatured β-lactoglobulin and κ-casein in the range pH 4.0–5.5 by using SPR. The extent of individual interactions was correlated with the molecular properties of the glucans.

Material and Methods

Glucans

The α-glucans, gluc55730, gluc180, glucML1 and glucA were produced by the purified glucansucrase enzymes GTFO, GTF180, GTFML1 and GTFA from *L. reuteri* strains ATCC-55730, 180, ML1 and 121, respectively. Production and isolation of the α-glucans have previously been described.[7,22,23]

Milk Proteins

β-lactoglobulin and κ-casein were purchased from Sigma-Aldrich (St. Louis, MO, USA). β-lactoglobulin (5 gL^{-1}) was heat-denatured (85ºC, 15 min) in sodium phosphate (50 mM, 30 mM NaCl, pH 6.8).

Surface Plasmon Resonance

Material

Sensor chip CM4, amine coupling kit (1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and ethanolamine hydrochloride), HBS-EP (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 150 mM NaCl, 3 mM etylenediaminetetraacetic acid (EDTA), 0.005 % surfactant P-20, pH 7.4) were used. All reagents and chips were purchased from GE Healthcare (Uppsala, Sweden).

Immobilisation of Milk Proteins

Each of the ligands κ-casein, native and denatured β-lactoglobulin were immobilised (Biacore T100; GE Healthcare, Uppsala, Sweden) in three separate flow cells, while the fourth was used as reference and underwent the same treatments, but without ligand. The ligand (50 μg mL^{-1} in 10 mM sodium acetate, pH 3.8) was immobilised to the carboxy methylated dextran surface on sensor chip CM4 by amine coupling chemistry. Briefly, the CM4 chip surface was activated by 8-min injection of a 1:1 (v/v) mixture of 0.1 M NHS and 0.4 MmM EDC followed by 7-min ligand injection and completed by 7-min injection of 1.0 M ethanolamine-HCl, pH 8.5 for blocking. κ-casein and denatured β-lactoglobulin were immobilised using Immobilisation Setup Wizard (Biacore T100 Control Software) while native β-lactoglobulin was immobilised manually by several 510 s injections until no further increase in resonance units (RU) was achieved. Throughout the immobilisation running buffer was HBS-EP and the flow rate 10 μl min^{-1} at 25ºC. The resulting immobilisation levels were 1130–1180 RU for native β-lactoglobulin, 3040–3060 RU for

denatured β-lactoglobulin and 3050–3900 RU for κ-casein. RU is an arbitrary unit reflecting the mass of ligand added to the sensor surface.

SPR Assay Procedures

The interaction of glucan (0.25, 0.50 and 1.0 mg mL^{-1} in 10 mM sodium acetate, 70 mM NaCl, 0.005 % surfactant P-20) was evaluated for each protein individually at pH 4.0, 4.5, 5.0 and 5.5 at 25ºC. Ionic strength was adjusted by addition of up to 100 mM, assuming full dissociation of acetate. Glucan was injected (association) for 120 s, followed by 90 s dissociation with the above acetate buffer and 120 s regeneration period with HBS-EP at a flow rate of 30 μl min^{-1}. The binding level was measured after 115 s of association. Each glucan sample was analysed in triplicates with each protein ligand including blanks (acetate buffer) and the experiment was done on two separately prepared sensor chips.

Data Processing

Double reference subtraction was used for all data.[25] In brief, binding response to the reference flow cell, i.e. non-specific binding, was subtracted from the binding response to the active flow cell, i.e. protein flow cell. This was followed by a subtraction of a blank sample injection. Each experiment was repeated twice at different sensor surfaces. Samples for which the non-specific binding exceeded 85 % of the binding in the active flow cell and samples for which binding was not observed in both runs were excluded. Mean values were calculated from different runs at the same sensor surface.

Results and Discussion

The binding levels of glucans gluc55730, gluc180, glucML1 and glucA (all at 0.25, 0.5 and 1.0 mg mL^{-1}) to native β-lactoglobulin, denatured β-lactoglobulin and κ-casein were screened at pH 4.0, 4.5, 5.0 and 5.5. As expected, the majority (about 75 %) of the binding experiments gave increasing RU values with increasing glucan concentration. However, some glucans, especially gluc55730 at unsystematic pH values, exhibited decreased binding at 1 mg mL^{-1}, perhaps due to aggregation of glucan molecules (data not shown). The binding levels are therefore presented for the 0.5 mg mL^{-1} glucan samples (Figure 1). The binding kinetics did not fit to single exponentials and could not be fitted to a simple 1:1 binding model most probably because multiple binding sites reside in the glucans as well as in the proteins. As a consequence, binding kinetics parameters and binding constants were not obtained. The binding response varied with glucan, protein and pH as exemplified in the representative sensorgrams (Figures 1, 2 and 3). The gluc180 and glucA showed the lowest binding response and saturated the denatured β-lactoglobulin surface shortly after start of the association, whereas glucML1 gave higher binding and its association curve only levelled slightly off at the end of the association phase (Figure 1). A similar pattern was observed for gluc55730 (Figure 2) at pH 5.0 and 5.5, but at pH 4.0 there were no signs of saturation of the surface under the association phase. This differs from the sensorgrams obtained previously for EPS from *Lactobacillus sakei*, *Lactobacillus plantarum* and *Lactobacillus salvarius* and milk proteins,[15] where the binding curves started to reach a plateau at the end of the association phase.

pH optima

The pH optima of the interactions seem to depend on the protein as well as the glucan. Thus native β-lactoglobulin showed best binding at pH 4.5–5.0, at low levels with glucML1 and at high levels with gluc55730, and thus the interaction seemed more favourable at pH values below the isoelectric point (pI) of 5.2.[26,27] β-lactoglobulin forms aggregates (i.e. octamers) at the investigated pH values, while the state of oligomerisation changes to dimers at pH values above 5.5 or to monomers at pH below 3.5,[12] which could account for the pH dependence of the glucan binding capacity. The gluc55730 thus interacted at pH 5.5; but no glucan binding, however, to native β-lactoglobulin was observed at pH 4.0. This was unexpected considering earlier data on HoPS binding,[15] showing relatively low binding of HoPS at pH 4.0 and none at pH 4.5–5.5 under the same conditions. The low binding levels of glucML1 and the lack of binding of gluc180 and glucA, however, are in agreement with the previous study[15] and may be due to the globular, rigid and hydrophobic structure of native β-lactoglobulin.[12] Such different behaviour in interacting with native β-lactoglobulin suggests that binding also depends on structural properties of the glucans used. When immobilising proteins onto the sensor surface, the molecular state of the proteins may be affected, which should be taken into account when interpreting the results.

As anticipated, the binding to β-lactoglobulin changed upon denaturation. The free thiol group of β-lactoglobulin is exposed and will be more susceptible to react with other compounds. Also, hydrophobic parts may be exposed, and formed aggregates can result in changed interactions with other molecules.[28] The binding of glucans depend on pH and was strongest at pH 4, perhaps due to effects of the denaturation being more pronounced at lower pH. Still, the four glucans behaved very differently and their molecular properties appeared to exert a major influence on the binding even to denatured β-lactoglobulin.

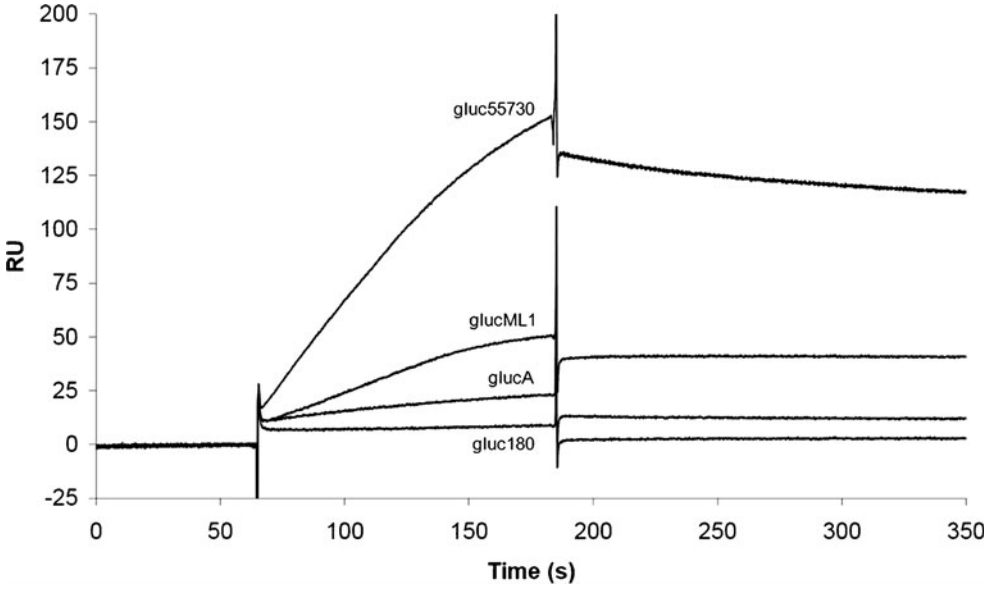

Fig. 1 Sensorgrams of the binding in resonance units (RU) of 0.5 mg mL^{-1} gluc55730, glucML1, glucA and gluc180 to denatured β-lactoglobulin at pH 4.0 measured by surface plasmon resonance. Association takes place at 65–185 s and is followed by the dissociation step

Binding to κ-casein depended much less on pH (Figure 3). κ-casein is an amphiphilic protein containing cysteine residues, which may participate in formation of intra- and/or intermolecular disulphide bonds.[29] When extracted from milk, κ-casein may be present as oligomers.[30] The thiol groups furthermore may interact with the surface of the sensor chip or be available for interactions with glucans. The isoelectric point of κ-casein vary greatly dependent on the form and the degree of phosphorylation as well as glycosylation. Values between 4.47 and 5.81 have been reported,[31] and it cannot be excluded that such modification of considerable heterogeneity may affect the binding interactions. However, this also likely reflects the situation normally present in milk fermentation and the overall binding capacity of the ensemble of κ-casein variants is of significant interest. The heterogeneity of the κ-casein may be reflected in the slight variation in glucan binding capacity of κ-casein at pH 4.0–5.5 (Figure 3). An overview of pH optima of the interactions of the individual proteins with the different glucans is given together with their linkage types and molecular weights (Table 1).

Relation Between Structural Properties of α-glucans and Their Protein Binding Ability

Despite the fact that all the present glucans originate from *L. reuteri* strains, their interactions with milk proteins at different pH values vary considerably (Figure 3), in a manner highly dependent on their structural properties. The gluc55730 and glucA possess predominantly α-(1,4) linkages, but in addition contain important amounts of α-(1,6)

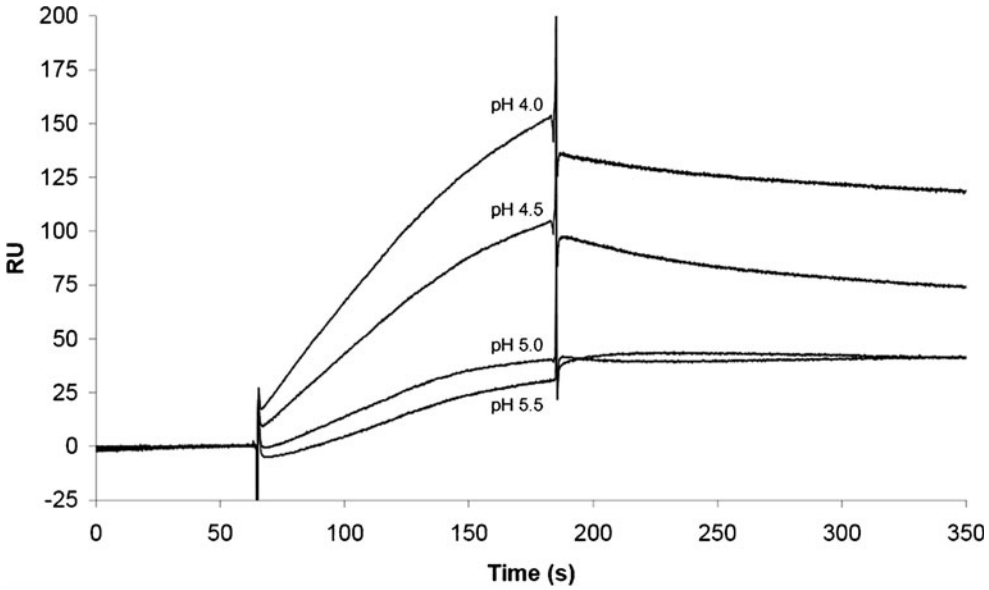

Fig. 2 Sensorgrams of binding in resonance units (RU) of 0.5 mg mL^{-1} gluc55730 to denatured β-lactoglobulin at pH 4.0, 4.5, 5.0 and 5.5 measured by surface plasmon resonance. Association takes place at 65–185 s and is followed by the dissociation step

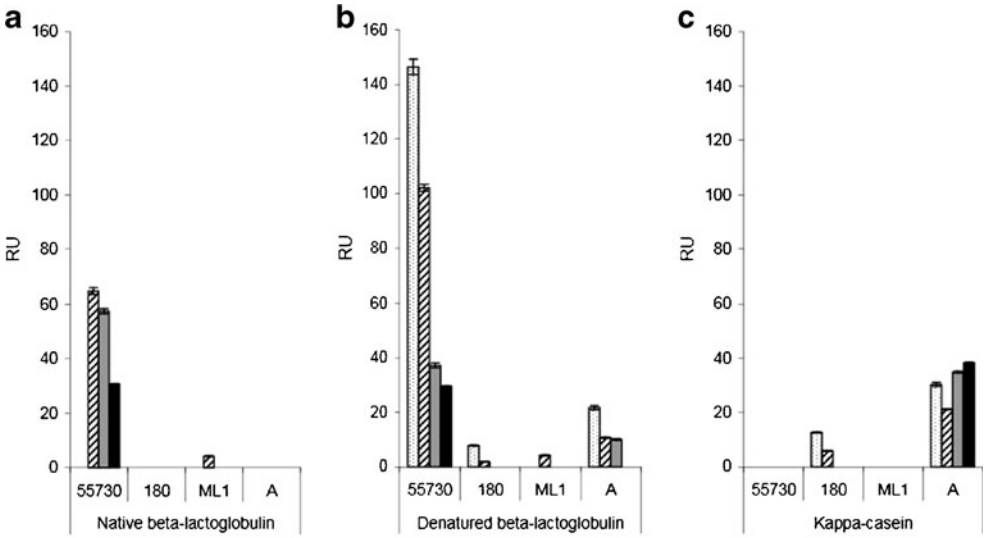

Fig. 3 Binding levels in resonance units (RU) after 115 s of association of glucans gluc55730, gluc180, glucML1 and glucA (0.5 mg mL^{-1}) to native β-lactoglobulin, denatured β-lactoglobulin and κ-casein at pH values 4.0 (dotted), 4.5 (crosshatched), 5.0 (grey) and 5.5 (black) analysed in triplicate measurements on the same sensor chip. Standard deviations are shown as error bars. Interactions that are not observed on both sensor chips are excluded

linkages and 4,6-disubstituted glucose residues (Table 1). Generally, gluc55730 showed the highest binding capacity with native and denatured β-lactoglobulin. The best binding of gluc55730 to native β-lactoglobulin was at pH 4.5 or 5.0 (pH optimum varies slightly between sensor chips), while no binding was observed for this glucan to native β-lactoglobulin at pH 4.0. For denatured β-lactoglobulin highest binding of gluc55730 was found at pH 4.0, which decreased with increasing pH, whereas no binding was observed to κ-casein in the analysed pH range. By contrast, glucA did not interact with native β-lactoglobulin, bound significantly albeit modestly to denatured β-lactoglobulin, and showed better binding level compared to the other glucans to κ-casein at all pH values. The high binding levels of gluc55730 or glucA to denatured β-lactoglobulin as well as to native β-lactoglobulin and κ-casein, respectively, suggest that α-(1,4) linkages generally favour protein binding.

The gluc180, despite its lower binding ability, showed the same binding pattern as glucA, i.e. no binding to native β-lactoglobulin, very low binding to denatured β-lactoglobulin at pH 4.0 and some binding to κ-casein at pH 4.0 and 4.5. Generally, binding levels of glucML1 were very low, with little binding to native and denatured β-lactoglobulin at pH 4.5–5.0 and 4.0–4.5 (the pH optimum differed between sensor chips), respectively, and no binding to κ-casein. The glucML1 thus shows some similarity to gluc55730 despite the pronounced difference in binding level. Both glucML1 and gluc180 contain α-(1,3), α-(1,6) linkages and 3,6-disubstituted residues, albeit in different ratios. Glucans with high proportions of α-(1,3) linkages are known to be more rigid and less soluble than glucans with lower amounts of α-(1,3) bonds,[32,33] which may affect the interactions with milk proteins. The correlation between binding levels and occurrence of α-(1,3) and α-(1,4) linkages indicated that HoPS with α-(1,4) linkages bind best to milk proteins regardless of molecular size and degree of branching (Table 1).

Even though gluc55730 and glucA have the same linkage types, they interact very differently with the different proteins, which possibly reflects the different content of α-(1,6) linkages. They have the same concentration of terminal glucose and disubstituted residues, but glucA has 26 % α-(1,6) linkages compared to 11 % in gluc55730. Similar differences in interactions were, despite their lower binding levels, observed for gluc180 and glucML1 containing 52 % and 10 % α-(1,6) linkages, respectively. The relatively low binding of gluc180 compared to glucA may be due to the rigidity imparted by the α-(1,3) linkages. The α-(1,6) linkages may promote interactions with κ-casein. From

Table 1 Overview of pH optima of binding of glucans gluc55730, gluc180, glucML1 and glucA to native β-lactoglobulin, denatured β-lactoglobulin and κ-casein as measured by surface plasmon resonance as compared with the linkage types (in %) and molecular weights reported previously

		55730	180	ML1	A
pH optimum	Native β-lg	**4.5–5.0**	–	4.5–5.0	–
	Denat. β-lg	**4.0**	4.0	4.0–4.5	4.0
	κ-casein	–	4.0–4.5	–	**All pH**
Linkage types	Terminal	9	12	18	9
	α-(1→3)	–	24	**47**	–
	α-(1→4)	**69**	–	–	**49**
	α-(1→6)	11	**52**	10	26
	α-(1→3,6)	–	12	26	–
	α-(1→4,6)	13	–	–	15
MW (10^7 Da)		2.8	3.6	0.8	4.5
References		([21])	([7,19])	([7])	([20,22])

Bold: highest binding level or percentage of linkage type, –: no binding

interactions with gluc55730 and glucML1, it appears that a high concentration of α-(1,6) linkages may hinder binding to native β-lactoglobulin, which is further reduced by α-(1,3) linkages and branching.

The molecular weight of the glucans may influence binding to native β-lactoglobulin and κ-casein. The glucA, which has the largest degree of polymerisation, showed the second highest binding level following gluc55730 to denatured β-lactoglobulin. Noticeably glucA showed the highest binding yield with κ-casein whereas gluc55730 bound most efficiently to native and in particular denatured κ-lactoglobulin. Remarkably, gluc180 exhibited the second best binding to κ-casein, suggesting that κ-casein more efficiently accommodated the glucan of higher molecular mass. The molecular mass may not directly affect the HoPS chain flexibility, but analysis of heteropolysaccharides of *Streptococcus thermophilus* showed that increased molecular weight of EPS resulted in increased viscosity, although the protein-EPS network did not seem to be affected.[34] The gluc55730 and glucML1 of the lowest molecular mass give most efficient binding to native β-lactoglobulin and glucan size may influence the higher level of κ-casein binding in addition to a high content of α-(1,6) linkages. In case of glucan binding to native β-lactoglobulin, the suppression by a high degree of α-(1,6) linkages may be more important than the size of the glucan for the binding level.

The glucML1 is more heavily branched (26 % disubstituted residues) than the three other glucans (12–15 % disubstituted residues), which may play a role for glucML1 having lowest binding levels in general. Branching may provide rigidity and hinder desirable orientation of the EPS backbone, and removal of side chains leads to increased flexibility.[8] Low conformational flexibility of the highly branched glucML1 may be responsible for the low binding to the milk proteins.

Though this study describes a simplified system, the new knowledge will be useful for applying glucans or EPS in fermented milk products. Interactions between glucans and milk proteins are reported to alter the microstructure of fermented milk products and to increase viscosity.[35] Thus, EPS-milk protein interactions, but also the intrinsic viscosity of the EPS itself,[36] are important for the texture. Intrinsic viscosity increases with increasing EPS molecular weight[34] and chain stiffness[36,37]. High molecular weight EPS may thus increase the viscosity by interacting with κ-casein and by contributing with its high intrinsic viscosity, while EPS with rigid chains may not interact with milk proteins and only contribute to texture solely by increasing the viscosity of the serum phase. Hence a mixture of EPS that interact differently with milk proteins would be favourable in manufacture of fermented milk products with higher viscosities[35,38]. The correlation between molecular properties of EPS, their interactions with milk proteins and texture of fermented milk should be further elucidated to gain insight in the origins of EPS functionality.

Conclusion

The interactions of EPS with native and denatured β-lactoglobulin and κ-casein are highly dependent on protein and glucan structure and to some degree on pH. Glycosidic linkage types, branching and molecular weight of the EPS affect interactions with milk protein. Generally, high binding levels are reached with HoPS containing α-(1,4) rather than α-(1,3) linkages, probably due to the superior conformational flexibility. Glucans with large amounts of α-(1,6) linkages show highest binding to κ-casein, whereas it seems to suppress interactions with native β-lactoglobulin. Apparently a higher degree of branching impairs interactions with milk proteins. Larger molecular weight seems to increase efficiency of binding to κ-casein. The interactions of glucans with κ-casein are not pH dependent, as opposed to denatured β-lactoglobulin which exhibited the best binding at pH 4.0 and native β-lactoglobulin at pH 4.5–5.0. These observations may be useful when applying EPS or glucans in fermented milk products to achieve an EPS-protein network resulting in higher viscosity.

Surface plasmon resonance analysis is demonstrated to be a useful emerging tool in characterising interactions between EPS and milk protein. Analysis of different HoPS and HePS of known structures both with individual milk proteins and in more complex systems should be continued to further develop the analytical tool and validates its practical application for manufacturing processes of yoghurts and fermented milk products.

Acknowledgements The authors would like to thank Danish Agency for Science, Technology and Innovation (FI), Ministry of Science, Technology and Innovation for partly financing the PhD studies of SKD. Darrell Cockburn is a DTU H.C. Ørsted postdoctoral fellow. BS thanks the Danish Council for Independent Research—Natural Sciences (FNU) for funding of the Biacore T100 instrument and a grant for studying protein polysaccharide interactions.

References

1. D.M. Folkenberg, P. Dejmek, A. Skriver, R. Ipsen, J. Texture Stud. **36**, 174–189 (2005)
2. P. Ruas-Madiedo, C. de los Reyes-Gavilan, J. Dairy Sci. **88**, 843–856 (2005)
3. S.A.F.T. van Hijum, S. Kralj, L.K. Ozimek, L. Dijkhuizen, I.G.H. Geel-Schutten, Microbiol. Mol. Biol. Rev. **70**, 157–176 (2006)
4. D. Bergmaier, C.P. Champagne, C. Lacroix, J. Appl, Microbiol. **95**, 1049–1057 (2003)
5. L. De Vuyst, F. De Vin, F. Vaningelgem, B. Degeest, International Dairy Journal **11**, 687–707 (2001)

6. M. Korakli, M. Pavlovic, M.G. Ganzle, R.F. Vogel, Appl. Environ. Microbiol. **69**, 2073–2079 (2003)
7. S. Kralj, G. H. Van Geel-Schutten, M. M. G. Dondorff, S. Kirsanovs, M. J. E. C. van der Maarel, L. Dijkhuizen, Microbiol.-SGM 150, 3681-3690 (2004)
8. R. Tuinier, W.H.M. van Casteren, P.J. Looijesteijn, H.A. Schols, A.G.J. Voragen, P. Zoon, Biopolym. **59**, 160–166 (2001)
9. M. Naessens, A. Cerdobbel, W. Soetaert, E.J. Vandamme, J. Chem, Technol. Biotechnol. **80**, 845–860 (2005)
10. I. Ayala-Hernandez, A. Hassan, H.D. Goff, R.M. de Orduna, M. Corredig, Int. Dairy J. **18**, 1109–1118 (2008)
11. M. Girard, C. Schaffer-Lequart, Food Hydrocolloids **22**, 1425–1434 (2008)
12. P. Walstra, J.T.M. Wouters, T.J. Geurts, *Dairy science and technology* (Taylor and Francis Group, Boca Raton, 2006)
13. L. Donato, F. Guyomarc'h, Dairy Sci. Technol. **89**, 3–29 (2009)
14. J.A. Lucey, M.I.C.H. Tamehana, H.A.R.J. Singh, P.A. Munro, Journal of Dairy Research **65**, 555 (1998)
15. L.N. Babol, B. Svensson, R. Ipsen, Food Biophys. (2011). doi:10.1007/s11483-011-9227-y
16. A. J. Tudos, R. B. M. Schasfoort, in *Handbook of surface plasmon resonance*, ed. By R. B. M. Schasfoort, A. J. Tudos (Cambridge, 2008), pp. 1–14
17. R.L. Rich, D.G. Myszka, J. Mol. Recogn. **21**, 355–400 (2008)
18. S.P. Muller-Renaud, D. Dupont, P. Dulieu, J. Agr. Food Chem. **52**, 659–664 (2004)
19. S. Marchesseau, J.C. Mani, P. Martineau, F. Roquet, J.L. Cuq, M. Pugniere, J. Dairy Sci. **85**, 2711–2721 (2002)
20. A.K. Thompson, H. Singh, D.G. Dalgleish, J. Agr. Food Chem. **58**, 11962–11968 (2010)
21. S.S. van Leeuwen, S. Kralj, I.H. Geel-Schutten, G.J. Gerwig, L. Dijkhuizen, J.P. Kamerling, Carbohydr. Res **343**, 1237–1250 (2008)
22. S. Kralj, G. H. Van Geel-Schutten, M. J. E. C. van der Maarel, L. Dijkhuizen, Microbiol.-SGM 150, 2099-2112 (2004)
23. S. Kralj, E. Stripling, P. Sanders, G.H. Van Geel-Schutten, L. Dijkhuizen, Appl. Environ. Microbiol. **71**, 3942–3950 (2005)
24. S.S. van Leeuwen, S. Kralj, I.H. Geel-Schutten, G.J. Gerwig, L. Dijkhuizen, J.P. Kamerling, Carbohydr. Res. **343**, 1251–1265 (2008)
25. D.G. Myszka, J. Mol. Recogn. **12**, 279–284 (1999)
26. A.M. Donald, Soft Matter **4**, 1147–1150 (2008)
27. M. Gottschalk, H. Nilsson, H. Roos, B. Halle, Protein Sci. **12**, 2404–2411 (2003)
28. T. Nicolai, M. Britten, C. Schmitt, Food Hydrocoll. **25**, 1945–1962 (2011)
29. H.E. Swaisgood, in *Advanced dairy chemistry*, ed. by P.F. Fox (Elsevier, London, 1992), pp. 63–110
30. L.K. Rasmussen, L.B. Johnsen, A. Tsiora, E.S. Sorensen, J.K. Thomsen, N.C. Nielsen, H.J. Jakobsen, T.E. Petersen, Int. Dairy J. **9**, 215–218 (1999)
31. J.W. Holland, H.C. Deeth, P.F. Alewood, Proteomics **4**, 743–752 (2004)
32. G.L. Cote, J.A. Ahlgren, M.R. Smith, J. Ind. Microbiol. Biotechnol. **23**, 656–660 (1999)
33. A. Shimamura, Y.J. Nakano, H. Mukasa, H.K. Kuramitsu, J. Bacteriol. **176**, 4845–4850 (1994)
34. E.J. Faber, P. Zoon, J.P. Kamerling, J.F.G. Vliegenthart, Carbohydr. Res. **310**, 269–276 (1998)
35. D.M. Folkenberg, P. Dejmek, A. Skriver, R. Ipsen, J. Dairy Res. **73**, 385–393 (2006)
36. P. Ruas-Madiedo, R. Tuinier, M. Kanning, P. Zoon, Int. Dairy J. **12**, 689–695 (2002)
37. P. Ruas-Madiedo, J. Hugenholtz, P. Zoon, Int. Dairy J. **12**, 163–171 (2002)
38. V.M. Marshall, H.L. Rawson, Int. J. Food Sci. Technol. **34**, 137–143 (1999)