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**Adherence interactions between milk proteins and human intestinal surface
layer components**

A thesis presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

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Abstract

Recent research suggests a number of food-derived proteins may be used as orally delivered functional components. The native structure is often vital to their activity and requires protection during the digestive process. Nutrient vehicles are used as protective envelopes and as a mechanism for targeting specific sites of activity, e.g. the small intestine. This study evaluated molecules which adhere to one or more *in vitro* models of three human intestinal surface layers. Successful candidates could then be incorporated into nutrient vehicles, promoting adhesion to the surface layers and resulting in prolonged retention of the active ingredient at the site of action or absorption.

To identify molecules that adhere to models of the intestinal surface, an adhesion protocol was developed to screen the proteome of whole milk, skim milk and whey for candidate proteins. Molecules adhering to model layers of the human gastrointestinal tract (intestinal epithelial cells, mucin or bacteria with the propensity to form a biofilm) were screened by SDS-PAGE analysis and identified by mass spectrometry and Western blot. The binding behaviour of selected proteins was further investigated by flow cytometry. The combined results showed that milk and whey proteins exhibit different binding affinities to the models of individual surface layers. α -Lactalbumin was found to adhere to a model of the intestinal epithelial cells, while β -lactoglobulin showed binding to the protective mucin layer. Lactoferrin and various components of immunoglobulins showed highest binding affinity to bacteria. Finally, IgM appeared to adhere to all three tested model layers of the human gastrointestinal surface. Least binding was observed to the intestinal epithelial cells in culture. The validity of the developed adhesion protocol was demonstrated by replicating adhesion of immune-related proteins, lactoferrin and immunoglobulins, to bacterial cells.

This work reveals new important characteristics of milk-derived proteins in their ability to adhere to models of the gastrointestinal surface. These may be further utilised in site-specific targeting of functional foods.

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... aus Lorbeeren macht man Kränze keine Betten.

- Steady Fremdkörper (Muff Potter)

I have been impressed with the urgency of doing

Knowing is not enough; we must apply.

Being is not enough; we must do

- Leonardo da Vinci

Meiner Familie.

Table of contents

Abstract	i
Acknowledgements	iii
Table of contents	vii
List of abbreviations	xiii
List of figures	xvii
List of tables	xxi
Introduction	1
Chapter 1 Review of literature	5
1.1 Physiology of the human gastrointestinal tract.....	7
1.1.1 The stomach	7
1.1.2 The small intestine - duodenum, jejunum and ileum.....	9
1.1.2.1 Absorption.....	11
1.1.2.2 Barrier	13
1.1.2.3 Signal recognition and transduction.....	13
1.1.2.4 Duodenum as designated site for targeted delivery of nutrients	13
1.1.3 The large intestine – caecum, colon and rectum.....	14
1.1.4 Physiological differences throughout the intestinal tract.....	14
1.1.5 Modes of interactions between food molecules and intestinal surfaces	16
1.1.6 Processes and structural changes during food digestion.....	17
1.2 Intestinal surface layers.....	19
1.2.1 Epithelial cell layer and glycocalyx.....	20
1.2.1.1 Cell culture of epithelial cells	23
1.2.1.2 HT29-MTX cells.....	24
1.2.1.3 Caco-2 cells.....	25
1.2.2 Mucin layer.....	26
1.2.2.1 Gel layer properties.....	26
1.2.2.2 Mucin molecules	28
1.2.2.3 Mucins and adherence interaction.....	31
1.2.3 Biofilms	32
1.2.3.1 Biofilm formation	33
1.2.3.2 Biofilm structure	36
1.2.3.3 Biofilm community	36
1.2.3.4 Well studied biofilm forming bacteria.....	38
1.2.3.5 Bacterial cellulose.....	39
1.2.3.6 Poly- β (1,6)-N-acetyl-D-glucosamine	41
1.3 Adherence mediating structures and transport systems.....	42
1.3.1 First generation of adhesives: mucoadhesives.....	43
1.3.2 Receptor-based interactions.....	43
1.3.3 Nanostructures	45
1.3.4 Liposomes.....	46
1.3.5 Anti-adhesive molecules.....	46
1.3.5.1 Milk components.....	46
1.3.5.2 Glycosides.....	47
1.4 Summary and Conclusions	48

1.5	Hypothesis and aims	49
Chapter 2 Materials and Methods.....		53
2.1	Introduction.....	55
2.1.1	Materials.....	55
2.1.2	Experimental work	59
2.2	Intestinal layer components	60
2.2.1	Mucin.....	60
2.2.1.1	Sepharose beads coated with mucin.....	60
2.2.1.2	Testing mucin coverage	61
2.2.2	Bacteria	63
2.2.3	Intestinal epithelial cells	63
2.2.3.1	Mono cultures	63
2.2.3.2	Co-culture.....	64
2.3	Test solutions	64
2.3.1	Whey and whey preparations.....	64
2.3.1.1	Acid whey	64
2.3.1.2	Whey using centrifugation	65
2.3.1.3	Protein quantification by Bradford assay.....	65
2.3.1.4	Protein quantification with DirectDetect	65
2.3.1.5	Partially digested whey	66
2.3.2	Protein labelling.....	67
2.3.2.1	DyLight594 labelling of whey	67
2.3.2.2	Rhodamine labelling	67
2.3.2.3	FITC labelling of proteins for Flow Cytometry.....	67
2.3.3	Milk.....	68
2.3.3.1	Digested skim milk	68
2.4	Adhesion assay.....	69
2.4.1	Mucin	69
2.4.2	Bacteria	71
2.4.2.1	Model 1 for bacterial biofilm: isolated biofilm components	71
2.4.2.2	Model 2 for bacterial biofilm: cell pellets from liquid culture.....	71
2.4.2.3	Whey sediment.....	72
2.4.3	Intestinal epithelial cells	72
2.5	Analysis of adhering proteins using the adhesion assay.....	74
2.5.1	Sodium dodecyl sulfate polyacrylamide gel electrophoresis.....	74
2.5.1.1	Chloroform-methanol-precipitation	74
2.5.1.2	Mucin beads	74
2.5.1.3	Bacterial pellets.....	75
2.5.1.4	Intestinal epithelial cells.....	75
2.5.1.5	Analysis.....	75
2.5.2	Western blot.....	76
2.5.3	Mass spectrometry analysis (ESI LC-MS/MS).....	78
2.6	Microscopy	79
2.6.1	Bacteria.....	79
2.7	Flow cytometry	80
2.7.1	Bacterial cell number and OD ₆₀₀	80
2.7.2	Titrating proteins onto bacteria.....	80
2.7.3	Titrating proteins onto intestinal epithelial cells.....	82

2.8	Statistics	82
Chapter 3 Adhesion assay development		83
3.1	Introduction.....	85
3.1.1	Hypothesis and aims	85
3.2	Results.....	86
3.2.1	Preparation of test solutions.....	86
3.2.1.1	Whey samples	86
3.2.1.2	Optimisation of in vitro gastric digestion	88
3.2.1.3	Effects of pepsin on mucin used to cover Sepharose beads.....	94
3.2.2	Coupling mucin onto Sepharose beads	94
3.2.2.1	Confocal laser scanning microscopy.....	94
3.2.2.2	2-D QuantKit.....	95
3.2.2.3	Nitrogen quantification by total combustion (LECO)	98
3.2.2.4	Wheat germ agglutinin tagging of mucin	98
3.2.2.5	Alcian blue (periodic acid Schiff).....	98
3.2.3	Design of the mucin bead adhesion assay.....	100
3.2.4	Optimisation and evaluation of the assay	100
3.2.4.1	Washing sequence and solutions.....	102
3.2.4.2	Sample resolution on SDS-PAGE.....	102
3.2.5	Final assay.....	109
3.3	Discussion	109
3.3.1	Whey and partial digestion	109
3.3.2	Sepharose beads with mucin.....	111
3.3.3	Adhesion assay.....	113
3.4	Conclusion	114
Chapter 4 Whey and milk proteins adhering to mucin covered beads.....		115
4.1	Introduction.....	117
4.1.1	Hypothesis and aims	119
4.2	Results.....	119
4.2.1	Milk.....	119
4.2.1.1	Skim milk	119
4.2.1.2	Whole milk.....	122
4.2.1.3	Mass spectrometric identification of adhering milk proteins.....	126
4.2.1.4	Western blot analysis	126
4.2.2	Isolated protein solutions	130
4.2.3	Whey and whey sediment	133
4.2.3.1	Description of whey sediment.....	133
4.2.3.2	Analysis of whey sediment	135
4.2.3.4	Treatments to remove the sediment from whey.....	135
4.3	Discussion	141
4.3.1	Adhering milk proteins	141
4.3.2	Isolated proteins	146
4.3.3	Whey.....	147
4.4	Conclusions.....	148
Chapter 5 Whey proteins adhering to human intestinal cells in culture.....		151
5.1	Introduction.....	153

5.1.1 Hypothesis and aims	154
5.2 Results.....	154
5.2.1 Adhesion assays	154
5.2.1.1 Band pattern and reproducibility.....	154
5.2.1.2 Quantification of SDS-PAGE gels using lane traces	158
5.2.2 Cell-adhesion of individual proteins analysed by Western blot	160
5.3 Discussion.....	165
5.3.1 Cell culture conditions.....	165
5.3.2 Adhesion assay.....	166
5.3.3 Western blot analysis	167
5.4 Conclusion	173
Chapter 6 Milk proteins adhering to biofilm producing bacteria <i>in vitro</i>.....	175
6.1 Introduction.....	177
6.1.1 Hypothesis and aims	178
6.2 Results.....	179
6.2.1 Development of the adhesion assay.....	179
6.2.1.1 Selection of model for bacterial biofilm components.....	179
6.2.1.2 Inclusion of fluorescent label.....	183
6.2.3 Use of the adhesion assay to identify bacterial adherent whey proteins.....	187
6.2.4 Identification of adhering milk proteins by mass spectrometry.....	189
6.2.5 Validation of adhering proteins using Western blot analysis	189
6.2.6 Direct visualisation of adhering proteins with fluorescent microscopy.....	191
6.3 Discussion.....	196
6.3.1 Adhesion assay	196
6.3.1.1 Impact of bacterial surface structures on the wash cycle.....	196
6.3.1.2 Adhesion assay.....	197
6.3.2 Mass spectrometry and Western blot analysis.....	197
6.3.3 Fluorescent Microscopy.....	201
6.4 Conclusion	202
Chapter 7 Adhesion of isolated proteins to human epithelial and bacterial cells.....	203
7.1 Introduction.....	205
7.1.1 Hypothesis and aims	205
7.2 Results.....	206
7.2.1 Degree of FITC labelling of isolated proteins	206
7.2.2 Binding of isolated proteins to human epithelial cells in culture.....	206
7.2.2.1 Titration curves	208
7.2.2.2 Analysis of area under the curve-progression: binding curves	208
7.2.2.3 Analysis of peak-progression along the X-axis: changes in signal intensity per cell.....	212
7.2.3 Binding of isolated proteins to bacterial cells.....	217
7.2.3.1 Initial titration curves.....	217
7.2.3.2 Analysis of area under the curve-progression: Binding curves	220
7.2.3.3 Analysis of peak-progression along the X-axis: changes in signal intensity per cell.....	224
7.3 Discussion.....	226
7.3.1 Binding of isolated proteins to human epithelial cells in culture.....	226
7.3.2 Binding of isolated proteins to bacterial cells.....	231
7.3.3 Lessons from flow cytometry.....	233

7.4	Conclusions.....	234
Chapter 8 General discussion		237
8.1	Objectives and hypothesis.....	239
8.2	Adhesion protocol.....	240
8.3	Adhesive proteins and mechanisms of binding	242
8.3.1	Immune related proteins	242
8.3.2	Caseins.....	248
8.3.3	Other binding proteins.....	249
8.3.4	Glycosylation of proteins	250
8.5	Achievements and limitations.....	252
8.6	Summary and conclusions	253
8.7	Future research.....	254
List of literature.....		259

List of abbreviations

90/10	Co-culture of Caco-2 and HT29-MTX cells in ratio 90:10
α -LA	α -Lactalbumin
β -LG	β -Lactoglobulin
BSA	Bovine serum albumin
CAPS	<i>N</i> -cyclohexyl-3-aminopropanesulfonic acid
CBB	Coomassie brilliant blue
CFU	Colony forming units
DL594	DyLight594
DMEM	Dubelco's modified Eagle medium
DMSO	Dimethyl sulfoxide
DTT	Dithiothreitol, Cleland's Reagent
ECL	Enhanced chemiluminescence
<i>E. coli</i>	<i>Escherichia coli</i>
ESI	Electrospray ionisation
EtOH	Ethanol
FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
Fuc	Fucose
Gal	Galactose
GalNAc	<i>N</i> -acetyl-galactosamine
GIT	Gastrointestinal tract
GlcNAc	<i>N</i> -acetyl-glucosamine
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
hr	Hour
HRP	Horseradish peroxidase
IEC	Intestinal epithelial cells

IgA/G/M	Immunoglobulin A/G/M
(IgG) hc	(Immunoglobulin G) heavy chain
kDa	kilo Dalton
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LF	Lactoferrin
LiCl	Lithium chloride
Man	Mannose
MeOH	Methanol
MFGM	Milk fat globule membrane
min	Minute
MTX	Methotrexate
MUC(2)	Mucin(2) protein
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NeuNAc / Neu5Ac	N-acetyl-neuraminic acid, sialic acid
NHS	N-hydroxysuccinimide
OD ₆₀₀	Optical density at 600 nm
PBS	Phosphate buffered saline
PBS-T	PBS with 0.05% Tween20
PBS 5.5	10:6 diluted PBS used during the wash cycle, pH 5.5
PIA	Polysaccharide intercellular adhesion, poly N-acetyl-glucosamine
PTS-domain	Proline, threonine, and serine rich region in mucin protein backbone
Rhd	Rhodamine
rpm	Revolutions per minute
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
<i>S. epidermidis</i>	<i>Staphylococcus epidermidis</i>
sec	Second

sIgA	Secretory Immunoglobulin A
SLB	Sample loading buffer
STD	Standard
TBS	Tris-buffered saline
TT	Tris-tricine
V	Volts
WGA	Wheat germ agglutinin

List of figures

Figure 1.1: Schematic representation of the human digestive tract. Adapted from Javadzahdeh and Hamedeyazdan [49]	8
Figure 1.2: Schematic representation of the mucus coverage of the gastrointestinal tract [60]. .	10
Figure 1.3: Schematic representation of the microbiota of the human gastrointestinal tract [18]	10
Figure 1.4: Three major functions of the intestinal epithelial cell monolayer. Adapted from Shimizu [66].....	12
Figure 1.5: Intestinal layers [196].....	22
Figure 1.6: Scheme of the human intestinal layers	22
Figure 1.7: Scheme of the development of a bacterial biofilm on a native tissue	34
Figure 1.8 A-B: Cellulose of different origins.....	40
Figure 1.9: Polymeric structure of pGlcNAc [417]	44
Figure 1.10: Different types of liposomes [418].....	44
Figure 1.11: Schematic showing the difference in passage of the gastrointestinal tract of unprotected active ingredients (left) and active ingredients delivered through a nutrient vehicle which is retained at the intestinal surface due to the action of anchor proteins (right)	51
Figure 1.12: Thesis structure.....	52
Figure 2.1: Outline of the final adhesion assay.....	70
Figure 2.2: Schematic showing different types of epithelial cell cultures and adhesion assay set-up.....	73
Figure 2.3: Relation between CFU.ml ⁻¹ and OD ₆₀₀ for three strains of bacteria	81
Figure 3.1: Protein band patterns of whey samples from different preparation methods.....	87
Figure 3.2: Pepsin digestion time course of whey	90
Figure 3.3: Efficiency of pepsin inhibition by bicarbonate and Pepstatin A	91
Figure 3.4: Pepsin digestion time course of skim milk.....	92
Figure 3.5: Micrograph of mucin covered beads and fluorescence signal	96
Figure 3.6: Micrograph of EtOH-amine blocked beads and fluorescence signal	96
Figure 3.7: 2D-Quantkit calibration curve.....	97
Figure 3.8: Calculated mucin concentration in coupling solutions.....	97
Figure 3.9: Calibration curve for AlexaFluor488-WGA measurements using a plate reader	99
Figure 3.10: Mucin concentration in different bead preparations.....	99
Figure 3.11: Outline of adhesion assay protocol	101
Figure 3.12: Adhesion assay between mucin-beads and digested whey	103
Figure 3.13: Adhesion assay between mucin-beads and digested whey; 12% acrylamide SDS-PAGE gel	105

Figure 3.14: Adhesion assay between mucin-beads and digested whey, concentrated by EtOH-precipitation	106
Figure 3.15: Adhesion assay between mucin-beads and digested whey, concentrated by CMP	107
Figure 3.16: Scheme of the final adhesion protocol including sample preparation.....	108
Figure 4.1 A and B: Representative models of the mucin network and glycoside side chain composition.....	118
Figure 4.2: Adhesion assay between mucin-beads and digested skim milk.....	120
Figure 4.3: Adhesion assay between mucin-beads and skim milk	121
Figure 4.4: Reproducibility of the adhesion assay between mucin-beads and digested or undigested skim milk	123
Figure 4.5: Adhesion assay between mucin-beads and whole milk	124
Figure 4.6: Western blot for β -LG on beads A after incubation of mucin-beads with skim and whole milk	127
Figure 4.7: Western blot for LF and pseudo milk (α -LA, β -LG, κ -CN, β -CN, α -CN) on beads A after incubation with skim and digested skim milk	129
Figure 4.8: Western blot for (A) β -LG and (B) α -LA on beads A after incubation with isolated β -LG and α -LA	131
Figure 4.9: Adhesion assay between mucin-beads and a β -LG and α -LA mix solution	132
Figure 4.10: Comparison whey or whey sediment observed during the adhesion assay.....	134
Figure 4.11: Whey sediment after each step of the wash cycle	136
Figure 4.12: Whey sediment after adding PBS salts individually	137
Figure 4.13: Decrease in whey sediment through incubation at 45°C.....	139
Figure 4.14: Western blot for LF on bacterial cell pellets after incubation with (A) whey and (B) sediment-free whey	140
Figure 4.15: Comparison of the adhesion assay (beads A) between mucin-beads and whey or sediment-free whey	140
Figure 4.16: Sediment in milks of different fat contents and degrees of processing.....	142
Figure 5.1: Time course adhesion assay between 90/10 co-culture and Rhd-whey (fluorescent scan).....	156
Figure 5.2: 30 min adhesion assay between 90/10 co-culture and Rhd-whey (fluorescent scan), triplicate sample	157
Figure 5.3: Lane traces from SDS-PAGE analysis of HT29-MTX whole lysate after different incubation times with Rhd-whey	159
Figure 5.4: Overview of Western blot analysis of Caco-2 cell fractions.....	161
Figure 5.5: Overview of Western blot analysis of 90/10 co-culture cell fractions	162
Figure 5.6: Overview of Western blot analysis of HT29-MTX cell fractions.....	163

Figure 5.7: Band density analysis of Figure 5.4 to Figure 5.6.....	164
Figure 5.8: Screenshot (assembled sections) of the PROSPER analysis, showing all determined potential enzymatic cleavage sites of β -LG [523].	168
Figure 5.9: Primary sequence of β -LG [533]......	171
Figure 6.1: Binding of whey proteins to GlcNAc covered Sepharose and negative control beads	180
Figure 6.2: Adhesion assay between <i>E. coli</i> Nissle and whey.....	182
Figure 6.3 A: Adhesion assay between bacterial cell pellets and Rhd-labelled whey.....	184
Figure 6.4: Density analysis of bands from Figure 6.....	188
Figure 6.5: Western blot analysis of all bacteria for LF, IgA and IgG heavy chain.....	192
Figure 6.6: Western blot analysis of all bacteria for IgM, XOR and β -LG	193
Figure 6.7: Density analysis of bands from Figure 6.5 and Figure 6.6.....	193
Figure 6.8: Micrographs of Rhd-skim milk or Rhd-whey binding to bacteria	195
Figure 7.1: Flow cytometry titration curves for α -LA binding to HT29-MTX cells.....	209
Figure 7.2: Flow cytometry titration curves for β -LG binding to Caco-2 cells.....	209
Figure 7.3: Flow cytometry titration curves for LF binding to Caco-2 cells.....	210
Figure 7.4: Flow cytometry titration curves for IgG binding to HT29-MTX cells	210
Figure 7.5 D-F (continued): Flow cytometry binding curves for all tested proteins and both cell types	214
Figure 7.6: Overview of binding of tested proteins (0.04 to 1 μ mol) to both cell types, as % total cells.	215
Figure 7.7: Overview of binding of tested proteins (indicated concentrations) to both cell types, as Δ geometric mean.....	216
Figure 7.8: Flow cytometry titration curves for α -LA binding to <i>S. epidermidis</i> 1457 M10	218
Figure 7.9: Flow cytometry titration curves for β -LG binding to <i>S. epidermidis</i> 1457 M10	218
Figure 7.10: Flow cytometry titration curves for β -LG binding to <i>E. coli</i> Nissle	219
Figure 7.11 G (continued): Flow cytometry binding curves for all tested proteins and bacteria	223
Figure 7.12: Overview of binding of tested proteins (0.05 μ mol) to all bacteria, as % total bacteria.	223
Figure 7.13: Overview of binding of tested proteins (0.05 μ m) to all bacteria, as Δ geometric mean.....	225
Figure 7.14: Schematic representation of the three suggested binding mechanisms for milk proteins adhering to (epithelial) cells in this study.	230

List of tables

Table 1.1: Milieu changes in the upper gastrointestinal tract induced by the ingestion of food .	15
Table 1.2: Physical and physiological changes throughout the intestinal tract	15
Table 2.1: Antibodies used for the Western blot analysis	77
Table 3.1: Development of a simulated duodenal fluid.....	93
Table 4.1: Mass spectrometric identification of selected adhering milk proteins	125
Table 4.2: Comparison of casein fraction ratios from Figure 4.3 (skim milk; n=1) and Figure 4.5 (whole milk; n=1) and those found in the casein micelle.	144
Table 5.1: Comparison of enzymes in the PROSPER database and those found in the human body and Caco-2 and HT29-MTX cells.....	170
Table 6.1: Bacterial binding proteins identified by LC-MS/MS	190
Table 7.1: Degree of FITC or Cy5 label at proteins for flow cytometry analysis (bacteria).....	207
Table 7.2: Degree of FITC label at proteins for flow cytometry analysis (cell culture).....	207
Table 8.1: Advantages and limitations of the analysis methods used in this thesis.....	241
Table 8.2: Analysis of advantages, limitations and development potential of the developed adhesion assay.....	243
Table 8.3: Comparison of identified adhesive proteins from Chapters 4 to 7	246
Table 8.4: Overview of milk protein glycosylation	251
Table 8.5: Summary of whey proteins adhering to one or all intestinal surface layer models in this thesis.....	255

