Adherence interactions between milk proteins and human intestinal surface layer components

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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.

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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	*N*-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
E. coli	*Escherichia coli*
ESI	Electrospray ionisation
EtOH	Ethanol
FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
Fuc	Fucose
Gal	Galactose
GalNAc	N-acetyl-galactosamine
GIT	Gastrointestinal tract
GlcNAc	N-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
S. epidermidis	*Staphylococcus epidermidis*
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

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