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Research paper

Lectins and bacterial invasion factors for controlling endo- and transcytosis of bioadhesive drug carrier systems[1]

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Abstract

Lectins are hemagglutinating proteins, found in all taxonomic groups of the plant kingdom and able to accomplish specific binding to membrane bound sugar moieties located at the cell surface of epithelial cells. Bioadhesive properties of lectins were quantified by their specific binding to Caco-2 cell monolayers. The differences between bioadhesion and mucoadhesion were described and two distinctive classes of lectins with different ways of intracellular trafficking were found. The concept of bioinvasion is explained by the invasion mechanism of *Yersinia pseudotuberculosis* after oral uptake. Bioinvasion is a new tool for oral drug delivery by receptor mediated endocytosis. Invasin, a 103 kD surface protein of Yersinia, was produced by biotechnology and covalently attached to model particles. We showed that the uptake of particles by MDCK cells can be significantly enhanced by functionalisation with invasin. Caco-2 cells do not express the receptors for Invasin ($\alpha_5\beta_1$ Integrins) and therefore are limited for such studies. © 1997 Elsevier Science B.V.

Keywords: Bioadhesion; Mucoadhesion; Lectins; Endocytosis; Transcytosis; Oral uptake; Drug delivery; Invasin; *Yersinia pseudotuberculosis*

1. Introduction

In the early 1980s, several scientific groups around the world pioneered the concept of bioadhesion to improve both local and systemic drug delivery [1–3]. Aim of bioadhesive drug delivery systems (BDDS) is their adherence to different tissue surfaces, i.e. usually epithelia representing absorption sites for drug molecules. As many epithelial tissues are covered by mucus, a visco-elastic hydrogel of 1–5% water-insoluble glycoproteins [4], systems capable of adhering to such surfaces are also referred to as mucoadhesive. Initially, bioadhesive drug delivery systems were expected to allow for: (i) a prolonged residence time at the site of drug action or absorption; (ii) a better localisation at a given target site, e.g. upper versus lower GI tract; and (iii) an intensified contact to the mucosa to increase the drug concentration gradient [5,6]. In particular the latter argument held some promise with respect to the improved delivery of peptide drugs, which usually do not easily pass through mucosal barriers.

Mucoadhesive polymers, either of natural or synthetic origin, show the remarkable ability to 'stick' to wet mucosal surfaces by non-specific, physico-chemical mechanisms, like van der Waals forces or hydrogen bonding. Although the term mucoadhesion is often used as a synonym for bioadhesion, it should be distinguished between the superimposed concept of bioadhesion and the special case of mucoadhesion. Mucoadhesion in a more strict sense refers just to one approach of realising bioadhesion, namely through adhesion to the mucus gel layer. Until today, this classic

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concept of mucoadhesion has not always been convincing, in particular not for the gastrointestinal route of drug delivery. This is partly due to the physico-chemical properties of the polymers used. A lot of polymers are at most weakly adhesive, if not non-adhesive at all, under conditions to be expected within the GI tract, e.g. abundant supply of water and varying pH. But two major problems, even for an intrinsically excellent mucoadhesive polymer, will remain: (i) rapid inactivation by soluble mucins, as well as food and other contents of the GI lumen; and (ii) the fast mucus turnover [7]. To overcome these problems, some new ideas have been recently brought up in order to achieve bioadhesion by approaches not based on mucoadhesive polymers.

2. Mucoadhesion versus cytoadhesion

This novel concept of bioadhesion deals with bioadhesives, which are able to bind directly to the cell surface by means of specific receptor mediated interactions. Such adhesion phenomena involve specific receptor-ligand interactions between complementary structures. Specific binding/adhesion is often based on lectin-sugar interactions. These lectin-sugar interactions can be classified by the following two categories: the first type of interaction involves the binding of an exogenous lectin, e.g. members of the large group of plant lectins, to endogenous sugars present on the cell surface. This binding is only possible if corresponding sugar moieties are available on the cell surface. The second type involves the binding of exogenous sugars to endogenous lectins. Whereby exogenous sugars are glycoproteins, glycolipids or artificial neoglycoproteins respectively neoglycolipids [8]. Endogenous lectins in this context are membrane bound lectins, usually called receptors (Fig. 1).

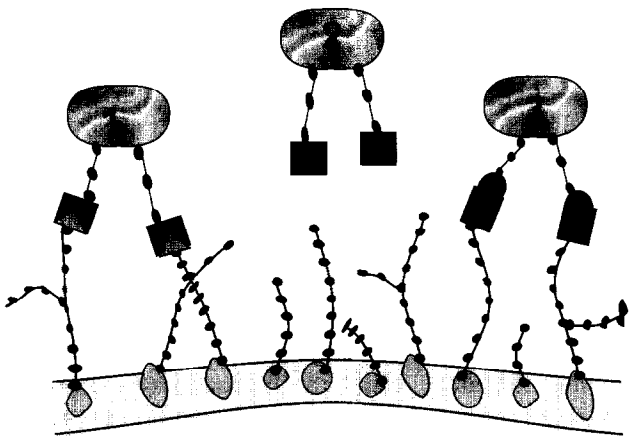


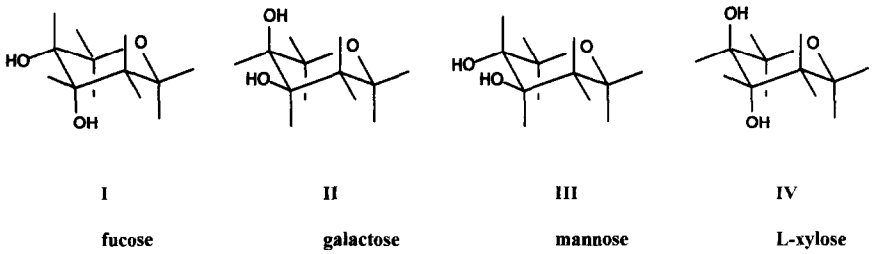
Fig. 1. Principal types of lectin-sugar interactions: 1. binding of exogenous lectins to endogenous sugars; 2. if no corresponding sugar is available, binding is impossible; 3. binding of exogenous sugars to endogenous lectins (taken from [75]).

This novel concept of bioadhesion, which should be referred to as cytoadhesion in order to distinguish it from mucoadhesion, is based on highly specific interactions, comparable with a specific antibody-antigen interaction. Compared with mucoadhesion, this has the advantage of a direct interaction with the cell surface rather than to mucus and may therefore offer some new perspectives for the controlled delivery of macromolecular drugs across epithelial absorption barriers. The mechanisms by which such cytoadhesives may help to overcome epithelial barriers are the following: (i) long-term fixation directly to the cell surface of cells, independent from mucus turnover; (ii) induction of and participation in specific vesicular transport processes (endo/transcytosis); and (iii) modulation of epithelial permeability by receptor-mediated opening of tight junctions.

2.1. What are lectins?

Over 100 years ago, the first plant lectin was described by Stillmark [9] which he discovered by working with extracts from castor bean (*Ricinus communis*). He obtained a fraction that agglutinated red blood cells. Ten years later, more and more of such substances from different plants were discovered, and the term haemagglutinin was proposed by Elfstrand [10]. Haemagglutinating proteins, which are not necessarily toxic, are found in all taxonomic groups of the plant kingdom. The discovery that some of the haemagglutinins are specific for blood groups led to the term lectin (Latin: legere = to select). The knowledge that lectins are able to bind some carbohydrates with high specificity, but are not members of the immunoglobulin family, and that in contrast to enzymes the lectin-sugar binding is reversible and does not alter the covalent structure of any of the recognised glycosyl ligands led to the general definition of Kocourek and Horejsi [11]. Lectins are not only found in plant kingdom, but also in vertebrates, as shown by Ashwell and Morell in 1974 [12,13]. These lectins are sometimes referred to as endogenous or reverse lectins. Lectins are also produced by bacteria or invertebrates [14]. However, plant lectins still represent the largest group of known lectins. Because lectins are a structurally diverse class of proteins—their only common feature is the ability to bind specifically and reversibly carbohydrates—it is difficult to group them together into distinct families [15]. However, based on the observation that lectin-reactive monosaccharides can be divided into four classes by the configuration of their 3- and 4-hydroxylgroups, it has been suggested to classify the lectins themselves analogously [16] (Fig. 2).

Lectins that bind fucose belong to group I, galactose and *N*-acetylgalactosamine to group II and glucose or mannose to group III. Up to now, no lectin has been



I fucose II galactose III mannose IV L-xylose

Fig. 2. Classification of lectins according to the configuration of C-3 or C-4 hydroxyl groups at the pyranose ring of specifically binding sugars.

identified, which is able to bind sugars of group IV (idose, gulose, L-glucose, L-xylose). A lot of lectins tolerate some variations at C-2 of the pyranose ring. Group III (mannose and glucose), contains one of the most intensively investigated lectins: concanavalin A (Con A) from the jack bean (*Canavalia ensiformis*). Other lectins of this group are those from peas, broad beans or lentils. Con A is a metalloprotein, that means that the binding of Ca^{2+} and Mn^{2+} is necessary for the sugar binding activity. Con A, as other lectins of this group, is known to interact with cell surface structures of different cells [17–22]. Wheat germ agglutinin [23,24], as well as the lectins found in potatoes [25], tomatoes [26], stinging nettle [27] and leguminosae are in the diverse group of *N*-acetylglucosamine-binding lectins, which belongs also to group III. In the group of *N*-acetygalactosamine and galactose-binding proteins (group II) are the so-called RIP II (ribosome inactivating proteins) lectins. Members of this historical class are ricin, the first observed lectin, abrin and the mistletoe isolectins. These lectins are dimeric glycoproteins, composed of two different subunits, A and B, linked by a disulphide bridge. Chain A is responsible for the toxicity, chain B for adhesion and cell entry. Other well-investigated lectins with the same sugar specificity are the soybean agglutinin and the isolectins obtained from *Phaseolus vulgaris* [28,29].

2.2. Lectins as bioadhesive molecules

In the last years, lectins have attracted the interest of pharmaceutical scientists because of their ability to accomplish specific binding to membrane bound sugar moieties located at the cell surface of epithelial cells. Another advantage of lectins is their generally good resistance to digestion within the GI tract. Several lectins have already been investigated in the light of possible pharmaceutical applications [30–32]. A particularly well investigated lectin is Phaseolus vulgaris haemagglutinin (PHA), for which a surprisingly high oral bioavailability of more than 10% after feeding to rats has been reported [33]. But PHA has to be considered as toxic, which will likely prohibit its use in pharmaceutical formulations. In contrast, tomato lectin (*Lycopersicon esculentum*) LEA can probably be regarded as safe, because of the widespread dietary consumption of raw tomato fruits. Therefore tomato lectin has attracted the interest of pharmaceutical scientists [34,35]. LEA belongs to the group of *N*-acetylglucosamine specific lectins. Other members of this group are wheatgerm lectin (WGA), which is also specific for *N*-acetylneuraminic acid (NANA) [36], and the smallest known plant lectin, obtained from stinging nettle UDA (*Urtica dioica*). The succinylated form of wheatgerm agglutinin WGAs has lost its ability to bind NANA, but not the specificity for *N*-acetylglucosamine [37]. The best inhibitory sugar for all four lectins is the tetramer of *N*-acetylglucosamine, but regardless of their very similar sugar specificity these lectins are much different in their molecular weight and isoelectric point (Table 1). Another interesting group of lectins are the isolectins from *Viscum album* (mistletoe lectins, ML). These lectins are discussed as anticancer drugs with immune modulatory effects and have already found approval for clinical application in tumour therapy [38,39]. The carbohydrate specificity for ML I, II and III decreased from ML I to ML III for D-galactose and increased for *N*-acetylgalactosamine.

2.3. Lectins as a key to control cellular adhesion, internalisation and routing

To compare the bioadhesive behaviour of the aforementioned two groups of lectins (specific for glucose or galactose, resp.), we investigated the binding and uptake properties to Caco-2 cells. The Caco-2 cell line is a well-known model to study epithelial transport mechanisms [40]. Caco-2 cells, derived from a human colon cancer, develop morphological characteristics of normal enterocytes when grown on plastic dishes or polycar-

Table 1
Molecular weight and isoelectrical point of *N*-acetylglucosamine specific lectins

Lectin	MW (kD)	pI	Specificity
UDA	9	9.5	$(NAcGluc)_4$
LEA	71	10	$(NAcGluc)_4$
WGA	41	8.5	$(NAcGluc)_4$; NANA
WGAS	36	4.5	$(NAcGluc)_4$

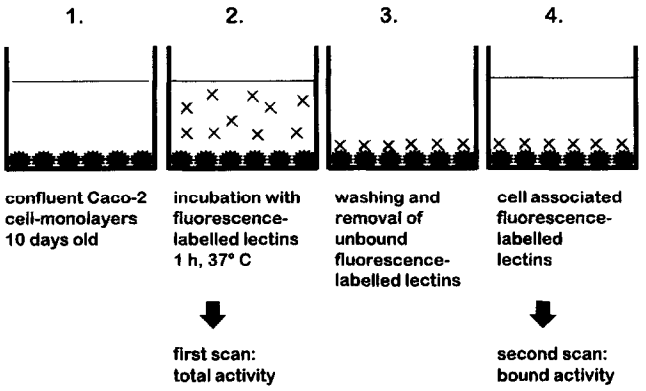


Fig. 3. Binding assay with fluorescently labelled lectins and confluent epithelial cell monolayers grown on 96 well plates.

bonate filters. For binding studies with fluorescently labelled lectins at 37 and 4°C the cells were cultured for 10–14 days on 96 well plates to confluency (Fig. 3).

Binding assays in presence of high amounts of bovine serum albumin (BSA) showed that the lectin-cell interaction for all lectins was not to be inhibited by BSA. Co-incubation with the corresponding sugar led to a marked decrease of binding. This means that binding is due to specific sugar-lectin, and not due to non-specific protein–protein interactions. A representative binding curve, showing total, non-specific and specific binding of tomato lectin, is given in Fig. 4.

After subtraction of non-specific binding, such data can be used to calculate binding capacity and affinity of the lectins, under the assumption of a one-ligand one-receptor model.

UDA, the smallest lectin, shows the lowest maximal binding capacity, followed by tomato lectin, which is the largest lectin in this group. WGA with a molecular weight of 41 kD shows the highest binding, but succinylation of WGA leads to a marked decrease of binding to the apical cell surface of Caco-2 cells. The affinity of all three lectins is in the same order ($1-2 * 10^{-6}$ M).

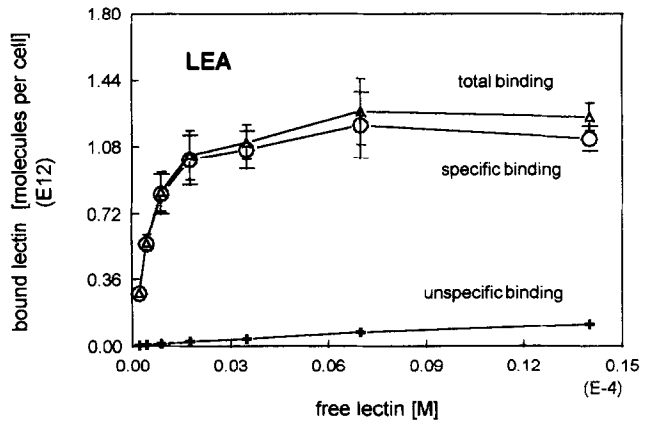


Fig. 4. Binding of tomato lectin (TL) at 37°C after 1 h in presence and absence of 10 mM $(NAcGluc)_4$.

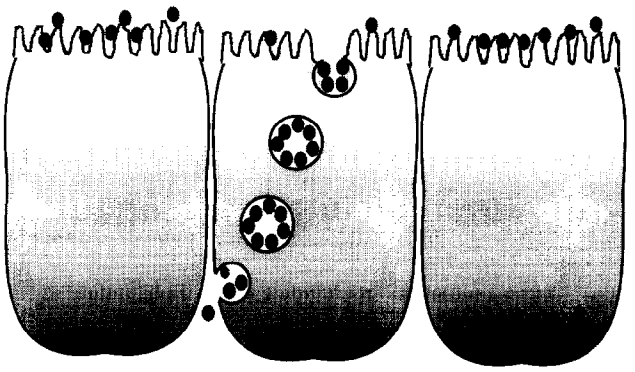


Fig. 5. Difference between surface binding and internalisation of molecules or particles to epithelial cells.

Binding of mistletoe isolectins used in the same concentrations as *N*-acetylglucosamine specific lectins was not saturable, but surely it is to look for saturation of binding by use of higher lectin concentrations. To differentiate whether lectin binding is restricted to mere adhesion to the apical cell membrane, or subsequently followed by internalisation into the cell through membrane derived vesicles (endocytosis, Fig. 5), the assays were also carried out at 4°C.

Difference of binding at low temperature is indicative for energy dependent uptake mechanisms. In the group of *N*-acetylglucosamine specific lectins, UDA and WGAs showed an increased binding at 4°C, while for LEA and WGA the binding was decreased at low temperature. Both, the increased signal at low temperature for UDA and WGAs and the decreased signal for LEA and WGA, are indicative for energy dependent uptake mechanisms, being involved in the interaction of these lectins with Caco-2 cells. The difference of binding behaviour at low temperature in this group of lectins depends on their different pathways in the cell. A possible explanation is that WGAs and UDA enter the cells by an endosomal/lysosomal pathway. These cellular compartments are characterised by a low pH (pH 4–5). As this acidification leads to quenching of the fluorescence label, one observes a decreased signal at 37°C compared with the stronger signal at 4°C. According to this model, lectins that are not quenched at 37°C, such as LEA or WGA, are obviously endocytosed by another, non-acidic pathway (Fig. 6).

In contrast to these results obtained with some *N*-acetylglucosamine specific lectins, no significant difference between 4 and 37°C with Caco-2 cells was observed. For *N*-acetylgalactosamine specific mistletoe lectins, our data suggest that the lectins chiefly bind to the cell surface and are not endocytosed (Fig. 7).

In summary, the lectins investigated show distinct differences in their binding to and interaction with the enterocyte like cell line Caco-2. All lectins bind to these cells by specific sugar-lectin interactions. However,

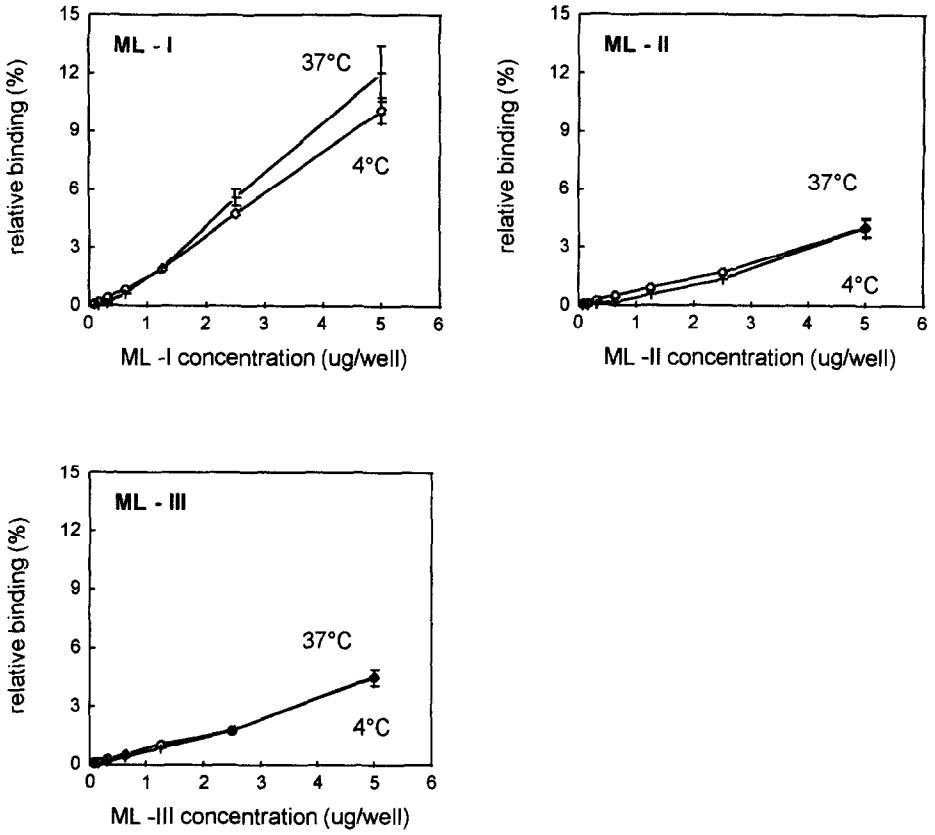


Fig. 6. Binding to Caco-2 cells of UDA, LEA, WGA and WGAs at 37 and 4°C.

while binding of the mistletoe lectins was temperature independent and suggested mere extracellular adhesion, the difference in binding at 4 and 37°C observed for WGA, WGAs, LEA and UDA is indicative that the adhesion of these lectins leads to invasion of the lectin into the cell by an energy dependent process like receptor mediated endocytosis along different intracellular pathways. With respect to some possible pharmaceutical applications, it may be concluded that lectins as specific cytoadhesives have the potential to control: (i) the adhesion; (ii) the internalisation; and (iii) the intracellular routing of lectin-coupled drugs or drug carrier systems.

3. From bioadhesion to bioinvasion

Besides mucoadhesive polymers and plant lectins, also some bioadhesive drug delivery systems based upon adhesive properties of bacteria have recently been investigated [41,42]. Bacteria are able to adhere to epithelial surfaces of the gastro-intestinal tract with the aid of their fimbriae [43]. The idea to use *E. coli* Type 1 fimbriae to prolong the transit time of a drug through the gut has also been filed as a patent [44]. Fimbriae are long, lectin-like proteins found on the surface of many bacterial strains. The presence of fimbriae has been found to be correlated with pathogenicity, e.g. adherence of *E. coli* to the brush border of the epithelial cell mediated by K99-fimbriae is a prerequisite for the subsequent production and uptake of the *E. coli* enterotoxin. Therefore, drug delivery systems based on bacterial adhesion factors could be an efficient means to enhance the adhesion of particulate drug carriers or hydrogels to epithelial surfaces.

3.1. Invasion of Yersinia species

Other bacteria not only adhere to the epithelia in order to live on outer surfaces of the host, such as e.g. the GI tract, but follow the strategy to invade into the host organism. The successful pathogen must gain entry to its host, find a suitable niche and multiply. Simply being able to adhere to a host cell is not enough for a subsequent invasion [45]. The pathogenic *Yersinia* species are transmitted via the fecal-oral route and enter and grow extracellulary in the reticuloendothelial system of birds and mammals. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are enteropathogens which gain access to the lamina propia from the intestinal lumen by entering into and passing through intestinal epithelial cells and cause a variety of illnesses, ranging from mild gastro-enteritis to mesenteric lymphadenitis [46]. The enteropathogenic *Yersinia* species have been

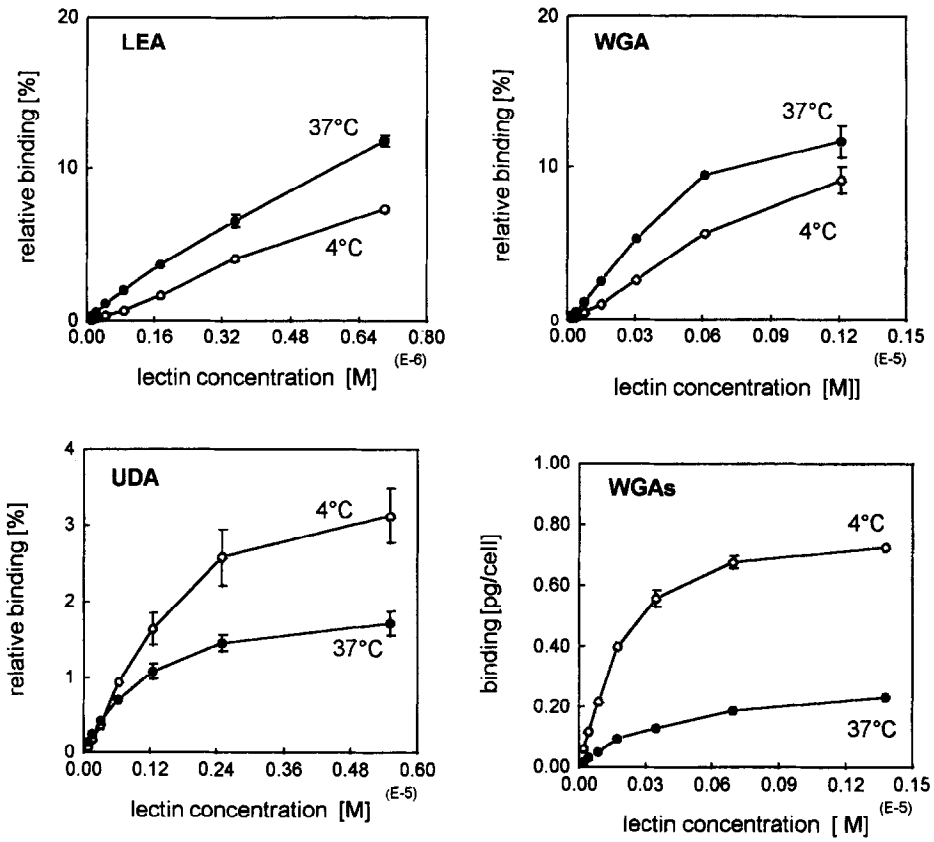


Fig. 7. Binding to Caco-2 cells of the three mistletoe isolectins at 37 and 4°C.

shown to interact productively with various cultured mammalian cell types, including nonphagocytic cells (epithelial, fibroblast and B cells) as well as phagocytic cells (macrophages) [47]. Directly after the attachment of the bacteria to the surface of the host cells, the bacteria are internalised by a process closely resembling phagocytosis.

Isberg et al. [48] isolated in 1987 cosmid clones containing 30 kB of *Y. pseudotuberculosis* DNA that converts the normally innocuous and not invasive *E. coli* K12 strain into an organism capable of entering cultured mammalian cells in a fashion similar to *Yersinia*. The genetic locus necessary to confer invasiveness, called *inv*, was shown to be a single region of 3.2 kB of *Yersinia* DNA. This locus allows *E. coli* strains to behave identically to the parent *Y. pseudotuberculosis* strain, with respect to both the efficiency of entry as well as the intracellular localisation of the bacteria in membrane-bound vacuoles. Further genetic and biochemical experiments by Isberg et al. showed, that the *inv* locus is a single transcriptional unit that encodes a single gene. The product of the *inv* gene is a 986 amino acid protein, called invasin, which is located on the outer membrane of the bacteria. Unlike invasin, adherence to epithelia mediated by fimbriae or pili is not directly followed by internalisation of the bacterium into the host cell.

These findings offer new approaches to develop bioinvasive drug delivery systems based upon the invasive properties of *Yersinia* and invasin, especially. The idea to use invasin as a vehicle for the introduction of molecules, particularly macromolecules, into a mammalian cellular host, either in vitro or in vivo, is subject of a patent by Isberg [49]. Logically, a pharmaceutical drug delivery system should comprise (a) a particulate drug carrier system in a size ranging up to 2–3 μm, which is the average diameter for *Yersinia* species, (b) the incorporated peptide drug or gene and (c) a particle surface which is modified with invasin or invasin derivatives. In summary, the entire drug delivery system resembles the bacterium *Yersinia* very closely (Fig. 8). With the knowledge of invasin and the invasin mediated endocytosis of *Yersinia* we also have the possibility to better understand the cell biology of bacterial invasion.

3.2. Receptors for invasin

The host cell receptors for invasin are multiple β_1 chain integrins. These integrins are members of the integrin superfamily of cell adhesion molecules (CAM). Members of this group of receptors are large, $\alpha\beta$ heterodimeric surface molecules that are able to bind

extracellular ligands as well as cytoskeletal components [50,51]. Integrins are involved in a wide variety of adhesive interactions. Each β chain is capable of associating with a number of different α chains, and a classification scheme has been proposed that subdivides integrins into subfamilies based on the particular β chain (called β_1, β_2, β_3 etc.). The six known VLA (very late antigens) proteins are members of one such subfamily of integrins that share the identical β_1 chain and have distinct α chains, which determine the substrate specificity of the heterodimer.

The $\alpha_2\beta_1$ chain integrin (VLA-2) is a receptor for collagen [52], while the $\alpha_5\beta_1$ chain integrin (VLA-5) is a receptor for fibronectin [53]. Some ligands, such as fibronectin, are able to bind multiple integrins [54], implying that different members of this receptor superfamily can recognise the same structural element presented by a particular ligand. The ability of ligands to be recognised by integrins is often associated with the presence of the tripeptide sequence Arg-Gly-Asp (RGD) in the substrate [55]. However, not all substrates for integrins contain this sequence and also other sequences may be important for recognition by integrins [56].

Invasin is able to bind a wide variety of cultured cell lines [57]. Many of these cell lines have large quantities of the β_1 chain, but no detectable α_5 chain. This means that the integrin $\alpha_5\beta_1$ cannot be the only invasin receptor, and affinity chromatography of crude bacterial detergent extracts demonstrated that integrins containing the subunit structures, $\alpha_4\beta_1$, $\alpha_5\beta_1$ and $\alpha_6\beta_1$ bound to immobilised invasin. β_2 chain integrins and $\alpha_2\beta_1$, integrin were not involved in cellular attachment to invasin [58].

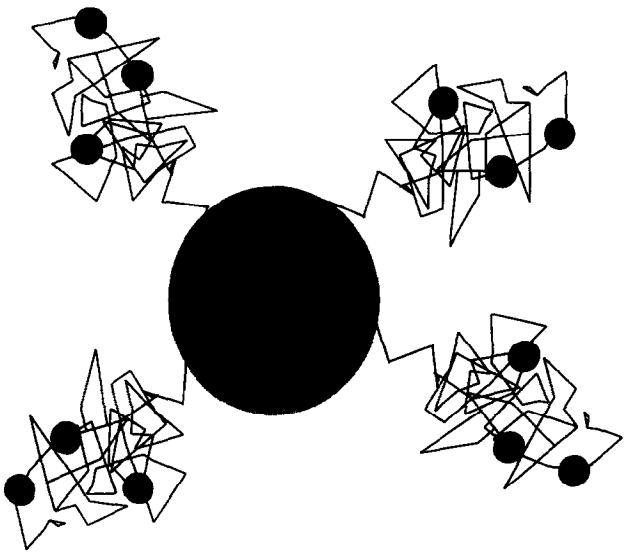


Fig. 8. Testsystem for bioinvasive drug delivery systems: carboxylated, FITC-labelled polystyrene nano-/microparticles (50 nm–3 μm) modified with a covalently attached INV-MBP fusion protein on the particle surface.

Leong et al. [59] isolated various antibodies against invasin. Six of these, which recognised epitopes within the last 192 amino acids of invasin, were capable to block cell attachment to invasin. Furthermore, deletion mutants of invasin carrying the last 192 amino acids retained their cell binding activity. These results showed that only the C-terminal 192 amino acids of invasin (INV-192) are necessary and responsible for the bioinvasive properties of the protein.

3.3. Biotechnological production of invasin

The testing of invasin for pharmaceutical purposes, such as invasin modified drug carrier systems, requires relatively large amounts of this protein. Invasin can readily be produced by genetic engineering. There are a variety of different types of plasmid vectors encoding different invasin derivatives (INV908-INV192) [60]. Maltose-binding protein (MBP)-invasin hybrid proteins can be purified in a single step by affinity chromatography using an amylose column. Several types of vectors designed for generating MBP hybrid proteins are commercially available. These vectors contain the *malE* gene, which codes for MBP, followed by a multiple cloning site, where a coding sequence at the 3′ end of the *malE* gene can be introduced [61]. These fusions are generally under P_{tac} control and thus can be induced with isopropyl-β-D-thiogalactopyranosid (IPTG). After affinity chromatography large amounts of pure and intact invasin can be obtained [62].

3.4. Exploitation of bioinvasive properties for pharmaceutical applications

The above described invasin has to be tested for the possibility to confer its naturally occurring bioinvasiveness to a pharmaceutical drug delivery system. If and when a drug delivery system can be modified with invasin derivatives, we would have a fantastic new tool to deliver macromolecules like proteins, peptides and genes orally into the cells of the gut, or even into systemic circulation. The design of a test system for a pharmaceutical invasin modified drug delivery system should meet some important requirements. The process of attachment of invasin to a particulate drug carrier or the cross-linking of a hydrogel has to be carried out under mild conditions to retain the specific biological activity of invasin. For binding and uptake studies, it is necessary to label invasin or the carrier system either radiochemically or fluorescently and the cell culture system should resemble the environment of human intestine.

One possible test system to assay invasin function has been described by Rankin et al. [61] Briefly, live, non invasive *S. aureus*, which expresses protein A on its surface, was incubated with anti-MBP serum. Protein A

Table 2
Genetic loci and names of bioinvasive proteins of some enteropathogenic bacteria

Bacterium	Genetic loci	Protein size	Name	Reference
EPEC (Enteropathogenic *E. coli*)	*eae*A	120 kD	Intimin	[69]
Listeria monocytogenes	*inl*A	80 kD	Internalin	[73]
Salmonella typhimurium	*inv*A-*inv*H >12 loci	n/a	Salmonella Invasins	[70,71]
Shigella flexneri	*ipa*-genes	17 kD (for ipgC)	ipgC-, ipaB-ipaC-Invasin	[74]
Yersinia pseudotuberculosis	inv	103 kD	Invasin	[48]

binds to the F_c region of IgG, and the result is the directional attachment of the antibodies on the bacterial surface, with the antigen binding sites facing outward. The bacteria were incubated with the purified MBP-INV hybrid protein to be tested. The hybrid can be attached via the anti-MBP serum, leaving the invasin-derived amino acids decorating the surface of the bacterium. This invasin coated *S. aureus* can now be added to epithelial cell cultures, incubated and treated with antibiotics. The antibiotics kill extracellular bacteria and surviving intracellular and bacteria can be counted after cell lysis and plating on agar.

Another test system uses commercially available, FITC-labelled, carboxylated nano-/and microparticles in various sizes [63]. Invasin can be covalently attached to these particles using standard methods [64]. Subsequently, the invasin modified particles can be incubated with epithelial cell monolayers. The remaining fluorescence after incubation and washing corresponds to particles that are either bound or taken up by the cells. To differentiate between particles which are taken up by the cells and particles which are only bound, it is possible to add Cytochalasin D to the incubation medium, which inhibits actin polymerisation and thus, endocytic uptake of particles [65]. Alternatively, anti-integrins or anti-β_1 integrin antibodies can be employed to block the integrin receptors for invasin. Furthermore, with the help of Confocal Laser Scanning Microscopy (CLSM) particles inside the cells can be readily visualised.

The Caco-2 cell monolayer system represents a widely used model for the human enterocyte. However, recent reports showed by indirect immunofluorescence staining that the invasin receptor $\alpha_5\beta_1$ integrin is not being expressed on confluent, differentiated Caco-2 cell monolayers [66]. These observations are in line with our experiments: Invasin modified particles with a diameter of 0.5 μm were not taken up by Caco-2 cells. The same particles applied to MDCK cell monolayers (canine epithelial kidney cells) showed a 2–3 fold enhanced binding and uptake in comparison to MBP modified particles as control [67]. Currently, mammalian cell infection by *Yersinia* is being investigated in vitro using non-intestinal cells such as HEp-2, CHO, and HEC1B, [68].

3.5. Other enteropathogenic invasive bacteria

Table 2 shows a listing of some important enteropathogenic bacteria and their surface proteins to attach and invade epithelial cells. A variety of virulent *E. coli* strains, associated with enteropathogenic and enterohemorrhagic diseases, encodes genes with high sequence similarity to a region of *inv* [69]. The best characterisation of these invasin-related proteins is the *eae* gene product from enteropathogenic *E. coli* (EPEC). This 102 kD protein facilitates adhesion of the microorganism to the apical surface of epithelial cells and is associated with actin aggregation and loss of microvilli at the site of bacterial adhesion.

Salmonella species are able to penetrate the epithelial barrier of the small bowel and translocate to submucosal regions. Genetic analysis of *Salmonella* entry into cultured cell lines indicates that there are at least two independent multigenic loci that encode factors critical for bacterial internalisation [70]. Galan and Curtiss [71] identified eight loci (*inv*A-*inv*H), which were defective for uptake after genctic lesions. Thus, there are at least 12 genes that influence the internalisation in *Salmonella* species. These findings stand in sharp contrast to the *Yersinia pseudotuberculosis* invasin, in which binding and entry are defined by a short region of a single protein.

Listeria monocytogenes is a gram-positive bacterium that causes systemic diseases in immunocompromised hosts after oral ingestion of contaminated foodstuff [72]. Insertion mutants in the *inl*A locus affect the production of an 80 kD surface membrane protein called internalin and after introducing a molecular clone harbouring *inl*A into the normally avirulent, non-invasive species *Listeria innocua*, an internalisation by Caco-2 cells was observable.

Besides Invasin, Internalin from Listeria monocytogenes seems to have bioinvasive properties as well. Bioinvasive proteins (Table 2) of Salmonella and Shigella are multi-enzyme complexes and have to react before the final enzyme complex is capable to invade.

4. Conclusion

Compared with from where it started in its beginnings, the concept of bioadhesive drug delivery systems has since undergone a number of remarkable changes. First, in contrast to the non-specific 'sticking' of some mucoadhesive polymers to the mucus gel layer covering the surface of various epithelial tissues, the use of cytoadhesive ligands (e.g. lectins) which adhere to molecular structures of the apical epithelial cell membrane by specific, receptor-mediated interactions promises the realisation of long-lasting bioadhesion independent of mucus turnover and non-productive interactions with other gut contents. Secondly, the specific interaction of such cytoadhesive ligands is not restricted to merely their fixation on the outer surface of the epithelium concerned, but instead can also transmit a biological signal triggering endocytotic uptake and transport by epithelial cells. Controlling receptor-mediated the endo- or transcytosis, however, opens several new perspectives for the controlled delivery of macromolecular drugs, such as large polypeptides or polynucleotides. Thirdly, specific bioadhesion implicates a shift from using well-known, inert and pharmaceutical approved polymers to the investigation of relatively unknown and moreover biologically active compounds as possible drug delivery adjuvants. This also means that such type of bioadhesive drug delivery systems can not be developed easily by some out-of-the-shelve technology. Instead, this novel concept of bioadhesive systems requires the thorough scientific investigation of both their biological and technological basis.

Whether some native plant or bacterial lectins, or derivatives thereof, may once be used in drug formulations indeed, will certainly not only depend on their efficacy, but also on their safety. Although the latter aspect will have to be addressed by separate investigations, the prospects for those lectins that are regularly ingested with our daily food should be very good. Otherwise, artificial non-toxic lectin derivatives can possibly be produced by means of genetic engineering. In order to fully understand the structure-activity relationship of lectin-cell interactions it is presently also important to study those lectins that are known to be toxic.

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