Cell. Mol. Life Sci. 62 (2005) 3002–3013
1420-682X/05/243002-12
DOI 10.1007/s00018-005-5228-7


Cellular and Molecular Life Sciences

Review Article

# Anti herpes simplex virus activity of lactoferrin/lactoferricin – an example of antiviral activity of antimicrobial protein/peptide

**H. Jenssen**

University of British Colombia, Centre for Microbial Diseases & Immunity Research, Room 232, 2259 Lower Mall Research Station, Vancouver, BC, V6T 1Z4 (Canada), Fax: +1 604 827 5566, e-mail: jenssen@cmdr.ubc.ca

Received 25 May 2005; received after revision 24 August 2005; accepted 6 September 2005
Online First 2 November 2005

**Abstract.** One of the most common viral infections in humans is caused by the herpes simplex virus (HSV). It was first effectively treated in the 1970s with the introduction of acyclovir, which is still the most commonly used treatment. Naturally occurring antimicrobial proteins and peptides have also been shown to possess antiviral activity against HSV. This review will focus on the anti-HSV activity of one such protein, lactoferrin, and a small peptide fragment from its N-terminal domain, lactoferricin. Both components have been shown to effectively block entry of HSV into the host cell. In addition to blocking HSV entry, the peptides appear to have immune stimulatory activity, although this is still somewhat controversial. Mode of action studies and knowledge about the anti-HSV activity of lactoferricin have also been successfully employed in the design of new, more specific HSV blockers.

**Key words:** Herpes simplex virus; lactoferrin; lactoferricin; cationic peptide; heparan sulfate.

## Introduction

*Herpesviridae* is a large family of viruses containing more than 130 different members, with at least one for most of the animal species examined to date. Nine different human herpes viruses have been described, and they are divided into three subfamilies; *alpha*-, *beta*- and *gamma-herpesvirinae*, based on their biological characteristics [1]. The human herpes family causes a variety of clinically significant diseases, although most of them are self-limiting in immunocompetent individuals. The most widely studied human herpes viruses are the two *alphaherpesvirinae*, herpes simplex virus 1 and 2 (HSV-1 and HSV-2). They are the primary agents of recurrent facial and genital herpetic lesions, respectively [2–3]. The antiviral targeting of these two viruses will be the main focus of this review.

Initiation of HSV infection involves attachment of viral glycoprotein C (gC) and-/or gB to heparan sulfate (HS) on the surface of the host cell (fig. 1) [4–5]. HS functions as an attachment receptor both for HSV-1 and HSV-2, although other glycosaminoglycan (GAG) molecules such as chondroitin sulfate (CS) may be used in the absence of HS [6]. Low infectivity of HSV has been reported in cells deficient in GAG molecules [4, 7–8]. However, viral attachment to HS alone does not enable viral entry. The entry process also requires viral glycoprotein D (gD) interaction with one or more co-receptor molecules on the cell surface (fig. 1). These entry co-receptors are divided into three structural families [9]. HVEM (herpes virus en-

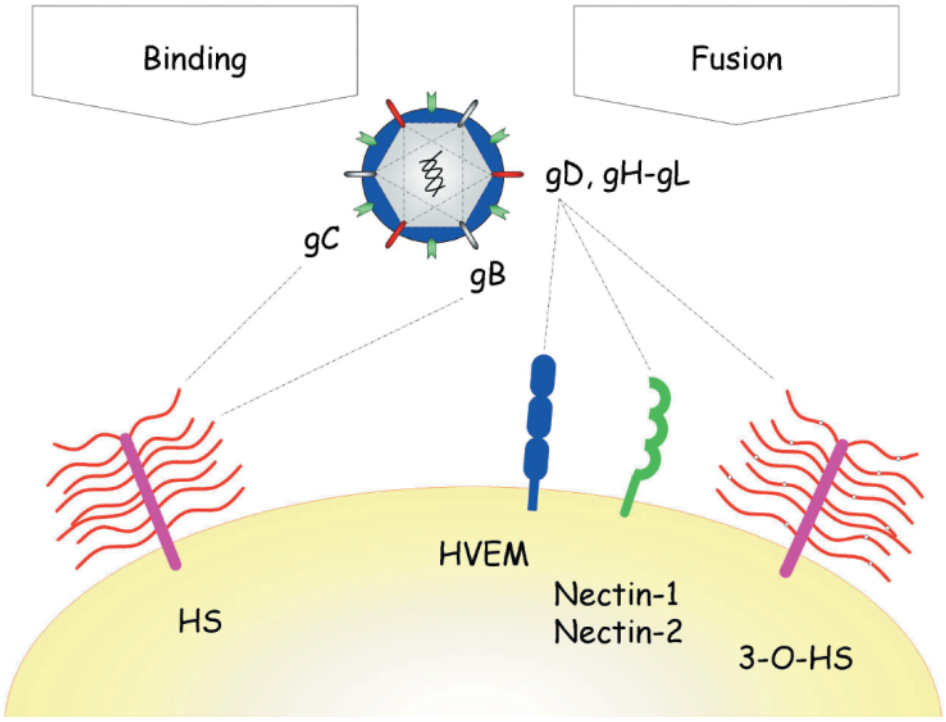


Figure 1. Cell surface receptors and viral ligands that participate in HSV entry. The viral envelope contains more than a dozen viral glycoproteins, but only five (gB, gC, gD, gH and gL) have been shown to participate in viral entry. Binding of virus to cells is mediated by the binding of gB or gC to HS chains on cell surface proteoglycans. This facilitates the binding of gD to one of its cell surface receptors. These include HVEM, nectin-1 and nectin-2, and specific sites in HS generated by certain 3-O-sulfotransferases. Binding of gD to any one of these receptors triggers fusion of the viral envelope with a cell membrane. This membrane fusion requires the action of gB and gH-gL heterodimers as well as gD and a gD receptor.

try mediator) [10], also called HveA [11], is a member of the tumor necrosis factor (TNF) receptor family. HveA is primarily located on lymphoid cells and mediates entry of both HSV-1 and HSV-2 [10, 12]. The second group of co-receptors consist of members of the poliovirus receptor-related (PRR) immunoglobulin superfamily; HveB/PRR2 [11], HveC/PRR1 [13] and herpes immunoglobulin-like receptor (HIgR) [14], now renamed nectin-2α, -1α and -1β, respectively [15–17]. Nectin-1α and -1β are broadly expressed on epithelial, fibroblastic, neural and hematopoietic cells, and mediate viral entry of both HSV-1 and HSV-2 [13], as well as cell-to-cell spread of HSV-1 [15]. Nectin-2α and its splice variant isoform, nectin-2δ (PRR2delta), mediates entry of HSV-2 [11, 16]. The third co-receptor family has only one member, the 3-O-sulfated heparin sulfate (3-O-HS). This modified HS molecule is generated by three different isoforms of D-glucosaminyl 3-O-sulfotransferase (3-OST); 3-OST-3 [18], 3-OST-5 [19] and 3-OST-6 [20]. It is broadly distributed on human cells and mediates efficient entry of HSV-1, but not HSV-2 [18–19]. HS therefore plays an important and complex role in promoting HSV-1 infection.

Viral entry is a result of fusion between the viral envelope and the host cell membrane, and requires four essential glycoproteins; gB, gD, and a heterodimer of gL and gH. The process results in release of tegument proteins and the viral capsid into the cytosol. Viral mutants lacking either of these glycoproteins manage to bind the cellular surface, but fail to exhibit fusion between the viral envelope and cell membrane [21–24].

There are two distinct ways for spread of wild-type HSV. The virus can be released from one cell and infect another cell, in accordance with the viral attachment and entry model (fig. 1). The virus can also be transferred across cell junctions between neighboring cells, referred to as cell-to-cell spread. The process is not fully understood, but by spreading from cell-to-cell across the tight junctions, HSV avoids neutralizing anti-HSV antibodies [25]. This mechanism appears to be especially important, since HSV establishes latent infections and may be reactivated periodically in the immunized host. By a mechanism known as cell-to-cell spread, host cell lysis is prevented and the virus evades the host's immune response.

Cell-to-cell spread involves a set of viral glycoproteins. Glycoproteins E and I have been shown to accumulate on lateral epithelial surfaces forming cell junctions, but not on lateral surfaces unrestricted by other cells. Mutant viral strains not expressing gE or gI display small plaques on a monolayer of human fibroblasts or epithelial cells [25–26]. Both gE and gI have been hypothesized to mediate HSV transfer across cell junctions by interacting with cell junction components [27]. Glycoprotein M has been shown to interact with both cellular receptors and the gE-gI complex at cell junctions, and thus facilitate cell-to-cell spread of the virus [28]. HSV remains cell associated at cell junctions throughout the transfer [29]. The viral glycoproteins gB, gD, gH and gL are all essential for successful cell-to-cell spread into the neighboring cell. Viral mutants lacking either of these glycoproteins can enter complementary host cells, but

cannot subsequently spread beyond the initially infected cell [21–23, 30].

The disease burden associated with viral infection is an escalating problem, with limited treatment regimes in place [31–32]. Few drugs have been approved for medical use against HIV and HSV in the last decades, despite tremendous research in this field. There is also considerable room for improvement in drug design, since most new drugs have similar targets as the drugs used today [33–35]. Current anti-HSV treatment specifically targets the viral replication process. The treatment is based on acyclovir (ACV) [36], a synthetic analogue of the nucleoside guanosin, or other pro-forms metabolized to ACV over time. The vast spectrum of these nucleoside analogues have been reviewed elsewhere [37].

## Antimicrobial proteins and peptides

The innate immune response involves among other things antimicrobial proteins and peptides [38]. Antimicrobial proteins are found in large amounts in all secretory fluids. Lactoferrin, lysozyme and collectins are among the most abundant secretion proteins in mammals [39–40]. Other proteins, including secretory immunoglobulin A, transferrin, mucin and histatins, are also important components of the innate immune system [41]. The antiviral activity of antimicrobial proteins is often related to opsonization of the pathogen, e. g. mannose-binding proteins interacting with HIV [42] and neutralization of influenza A virus by surfactant protein A [43]. Lactoferrin is known to work as an opsonin for bacterial clearance [44], but this activity has not been illustrated for viruses.

Antimicrobial peptides are produced by a wide variety of organisms as their first line of defense, so-called the innate immune strategy [38]. To date, hundreds of such peptides have been isolated [45], indicating their importance in the innate immune system [46]. Antimicrobial peptides are typically relatively short (12 to 100 amino acids), positively charged, amphiphilic and have been isolated from single-celled microorganisms, amphibians, birds, fish plants and mammals, including man [47–48]. The most prominent peptide structures are 2–4 β-strands, amphipathic α-helices, loop structures and extended structures [49–50].

Members from all of the four structural classes of antimicrobial peptides have been shown to inhibit viral infection. The spectrum of viruses that are affected primarily comprise the enveloped RNA and DNA viruses. In most cases it has been concluded that antiviral activity is exerted at a very early stage in the viral multiplication cycle, either by direct action of the peptides on the virus itself [51–52] or at the virus-cell interface [53]. It has also been demonstrated that antimicrobial peptides regulate multiple cellular genes [54], findings which support peptide stimulation of the cellular immune response [55]. A reasonable hypothesis is that the products of a subset of these peptide-upregulated genes are able to suppress endotoxic responses that lead to production of pro-inflammatory cytokines while upregulating other genes assist in resolving infections [56–57].

## Lactoferrin

Lactoferrin (Lf) is an 80-kDa multifunctional, monomeric glycoprotein [58] present in external secretions, especially milk, tears and saliva. The protein folds into two homologous globular lobes linked with an 11-amino acid α-helix [59]. Each lobe may reversibly bind one ferric ion [60]. Lf evolved several million years ago, and its importance as an antimicrobial protein is underscored by numerous conserved gene segments. The conserved regions namely comprise areas on the surface of the protein structure [61]. There is 69% sequence homology between bovine and the human Lf (bLf and hLf) [62–63]. Lf shares many structural and functional features with the plasma iron-transport protein transferrin [59]. Lf is quite resistant to tryptic digestion, resulting in partial survival following passage through the gastrointestinal tract [64]. The antibacterial activity of Lf has been well reviewed against a broad spectrum of bacterial strains [65–67]. Several immunological functions have been ascribed to Lf, although their detailed mechanisms remain unknown [68–73]. Lf has also been shown to inhibit tumor growth [74], fungal infections [75–76] as well as viral infections [77–89]. The antiviral activity of Lf has been demonstrated against both naked [87, 90–92] and enveloped viruses [79, 81, 84–86, 93–100]. This activity is exerted during an early phase of the viral infection. Lf from several species possesses antiviral activity towards different human viruses, although bLf is often reported to exhibit higher antiviral activity than hLf [83–84, 93, 101]. One of the main physiological functions of Lf is to bind iron. However, iron saturation does not appear to influence the antiviral activity [85–86, 88].

## Cellular targets and antiviral mode of action of Lf

Andersen et al. [55] showed that the antiviral activity of Lf not was improved by pre-incubation of Lf with HSV-1 or HSV-2 prior to infection, indicating that the antiviral activity of Lf must be exerted through interaction with a cellular target, rather than a target on the viral particle itself [55]. Conversely, Marchetti et al. [84–85] suggested that Lf prevents HSV entry in part by binding to the virus particles. However these mechanisms need not be exclusive, and may reflect the different experimental conditions. Electron micrographs have confirmed that

Lf must be located at the cell surface to exert antiviral activity against HSV [55]. These and others studies have also demonstrated that Lf remains at the cell surface after exposure [102], which explains the post-infection effect of Lf observed with the plaque reduction assay in Vero cells [55].

The antiviral mode of action of Lf has been described for several virus strains. Interaction between Lf and the host cell inhibits infection by hepatitis B virus (HBV) and HSV [80, 55]. In contrast, for infections with adenovirus, feline herpes virus (FHV-1), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infection, Lf exerts its antiviral activity by direct interaction with the viral particle [77, 96, 103–104]. In all cases studied, it appears that Lf exhibits its antiviral activity at an early phase in the infection process [55, 77–78, 80, 83, 96, 103–105].

Several viral pathogens has been shown to use host cell surface HS as an attachment receptor [106–107] during the infection process. Lf also binds HS [108] as a result of its high net charge and two N-terminal domains for GAG binding [109–111]. Viral entry of HSV-1 is effectively blocked by Lf [55], most likely as a result of Lf interaction with cell surface HS [81, 84, 112]. Anti-HSV activity of Lf has been investigated with several cell lines, both deficient in and expressing different GAG molecules at the cell surface. The results have shown that HS at the cell surface is important for Lf to exert antiviral activity [55, 112]. HSV-1 and HSV-2 differ in their interaction with HS [113], which may explain the different antiviral activity of Lf towards the two viruses [101].

Lf can effectively block viral entry when added to the cells prior to infection. Moreover, pre-incubation of the cells with Lf for 4 h prior to viral infection also does not affect the blocking of viral entry [55]. Significant amounts of Lf are internalized after 4 h, and Lf has been shown to exhibit antiviral activity only when present at the cell surface [55]. This may indicate that the cells are able to adapt a long-lasting antiviral immunity after exposure to Lf. Similar immunity to HSV infection, lasting for several hours, has also been reported for derivatives of dispirotripiperazine [114], interacting with HS.

Lf has mainly been localized at the cell surface of HS-expressing cells; localization shifts intracellularly in cell lines deficient in GAG expression [55]. This has been explained by the high affinity of Lf for HS [108]. Variation in the amount of Lf found on the surface of different HS expressing cells may be explained by a diverse expression pattern for HS; variation might also result from different affinities of HS to Lf resulting from diversity in the primary structure of HS on the individual cell types [115–116]. However, the antiviral activity of Lf cannot be fully explained by competition for viral attachment sites [117].

## Cellular uptake of Lf

Ji and Mahley [118] have illustrated that mutant CHO cells (pgsD-677) lacking HS bound much less Lf compared which wild-type CHO cells. The results suggest that Lf may bind directly to the low-density-lipoprotein receptor-related protein (LRP) in the absence of HS [118]. This may explain binding of Lf to cell surfaces in the absence of HS and other GAG molecules [55]. LRP has also been shown to act as an endocytosis-mediating receptor [119], and may thus explain how Lf is internalized in GAG-deficient cells [55].

A specific receptor for Lf (LfR) has been characterized on several mammalian cell types and tissues including monocytes [120], lymphocytes [121], liver [122–124] and the small intestine [125]. Cellular uptake of iron bound (holo) Lf in the small intestine has been linked to the LfR [126], implying that LfR has a higher affinity for holo- than apo-Lf (iron free). This may explain the differing amounts of apo- and holo-Lf found on the surface of Vero cells, even though they have the same affinity for HS [55].

Lf has the ability to interact with nucleolin at the cell surface [127]. Nucleolin is mainly expressed in the nucleus, but is shuttled to the cell surface and back to the nucleus over the cytoskeleton [128]. Independent of the presence of GAG molecules, Lf binds nucleolin specifically with medium affinity, through binding sites located in both the N- and C-terminal lobes of Lf [127]. Internalization of the Lf-nucleolin complex through vesicles of the recycling/degradation pathway, however, requires HS. Legrand et al. [127] have illustrated that this mechanism results in nuclear localization of Lf.

Experiments have demonstrated that hLf is internalized into cells even at 4 °C [55], a temperature at which the endocytic pathway should suffer considerable suppression [129]. The N-terminal region $G_1RRRR_5$ of hLf, identified as a nuclear localization signal (NLS) [130] is responsible for this type of Lf internalization. This sequence is also the heparin-binding site of Lf [131]. Internalization of Lf to the nucleus can therefore be blocked by heparin [132], although there is no evidence linking this internalization to HS on the cell surface. Energy-independent internalization has also been shown in GAG-deficient cells [55]. Small-angle scattering studies have demonstrated that both lobes of Lf undergo a substantial conformational change as a result of iron binding, consistent with closure of the inter-domain cleft [133]. Variation in the relative amounts of apo- and holo-hLf detected in GAG-deficient cells [55] likely indicates that energy-independent internalization through the NLS is influenced by the secondary structure of Lf.

Several internalization processes have been proposed and confirmed for Lf, including both a receptor-induced endocytic pathway. e. g. using LRP, LfR or nucleolin,

and/or an energy-independent entry involving NLS. Lf is a complex molecule and may use different internalization mechanisms, depending on its iron bound state and the experimental conditions.

## Intracellular targets for Lf

Even though Lf has numerous immune-stimulating properties [134–136], little is known about the actual mechanisms involved. Intracellular localization of Lf may result in regulation of host cell protein expression. Legrand et al. [127] have shown that Lf is transferred into the nucleus after interaction with the cell surface protein nucleolin. Comparable amounts of hLf have also been observed in the cytoplasm and in the nucleus by Andersen et al. [55]. It has also been reported that Lf binds a specific DNA sequence [137] and activates the transcription of interleukin (IL)-1β [138]. *In vivo* studies have also demonstrated an increase in serum levels of IL-18 and splenocyte production of interferon-γ and IL-12 upon orally administered of Lf [139]. These ILs have the ability to protect the host from infections caused by HSV [140].

## Fragments of Lf: antiviral cationic peptides

Two large fragments of bLf, the C-lobe [amino acid (aa) 345–689] and the N-lobe (aa 1–280), have been shown to inhibit HSV-1 infection, while a smaller part of the N-lobe (aa 86–258) was ineffective [141]. A small heparin-binding antimicrobial peptide (lactoferricin) has been isolated from the N-terminal domain of Lf, following pepsin treatment [142]. Bovine lactoferricin (LfcinB) is situated between residues 17 and 41 in bLf, while human lactoferricin (LfcinH) consists of two fragments, aa 1–11 and 12–47, connected by a disulfide bridge [143]. Reproduction of these results, however, has been difficult, and several recent publications have defined LfcinB as aa 17–42 [144], and LfcinH as a single fragment of aa 1–49 [145] (fig. 2). LfcinB features a loop region attributable to a disulfide bridge between residues 19 and 36, a region which is also found in the homologous region of LfcinH (aa 20 and 37) [143]. Both LfcinB and LfcinH create a surface-exposed amphipathic α-helical domain in Lf prior to pepsin digestion [146–148]. In addition, the larger LfcinH comprises a parallel β-sheet structure. After pepsin cleavage, LfcinB loses its α-helical domain and becomes a distorted antiparallel β-sheet [149], whereas LfcinH retains its α-helical domain but loses its β-sheet [145]. LfcinB has been shown to exert antiviral activity against HCV [82], HCMV [93], HIV [78], HSV-1 and HSV-2 [101, 108], while LfcinH has shown antiviral activity against HSV-1 and HSV-2, although this activity is lower than that of LfcinB [101]. The difference in secondary structure may explain the difference in antiviral activity [101, 108]. Several other β-sheet peptides have been described as potent inhibitors of HSV infection [150], while only melittin and magainin from the group of α-helical peptides have been reported to possess anti-HSV activity [151].

## Structural requirements for the antiviral activity of Lfcin

A set of LfcinH and LfcinB derivatives have been constructed and tested for their antiviral activity against HSV-1 and HSV-2 in an attempt to identify primary

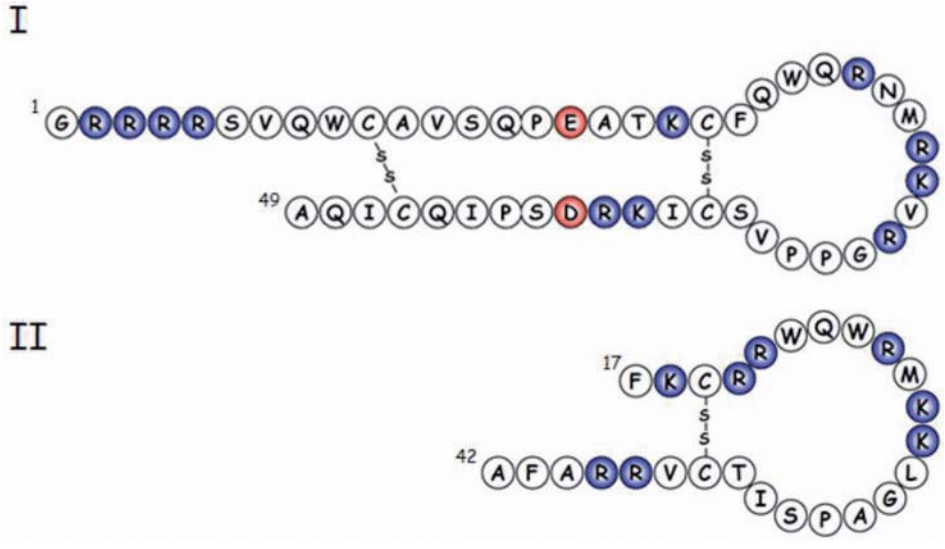


Figure 2. Primary structure projections of LfcinH and LfcinB. Primary structures of LfcinH (I), consisting of amino acids 1-49 and LfcinB, (II) comprising residues 17-42.

structure domains contributing to peptide antiviral activity [108]. Deletion of segments in the N- and C-terminal part of LfcinH gave the corresponding human analogue of LfcinB with no detectable antiviral activity [108]. This could be a result of structural changes due to the reduction in chain length and/or the reduction of the charge from 8.79 to 5.85 at pH 7.0, since antiviral peptides often are described as highly cationic [150–152]. The relationship between a peptide's net charge and its antiviral activity has been reported for both the Lfcin analogues and a set of short α-helical peptides [108, 152–153]. However, the spatial positions of the charged amino acids seem to be more important for antiviral activity than the actual net charge [108, 152].

Several types of charged molecules in the body have been shown to interact through electrostatic interactions with GAG molecules on the host cell surface [154–163]. Several Lfcin analogues and short α-helical peptides have also shown high affinity for HS and this affinity has in part been correlated with the peptides' net charge [108, 152]. Peptide affinity increased further if arginine was substituted for lysine [152, 164–166]. Even though LfcinH has a higher net positive charge than LfcinB, i. e. 8.79 and 7.84 at pH 7.0, respectively, the two peptides exhibit equal affinity for HS. Thus, other parameters influence peptide affinity for HS [108]. Aromatic amino acids have a minor influence on the peptides' HS affinity [108, 152]. However, specific GAG binding domains have been identified in both LfcinH and LfcinB. These domains involve the sequences $G_1RRRRS_6$ and $R_{28}KVR_{31}$ in LfcinH [109], and the whole sequence of LfcinB [110]. The widespread positioning of these elements in the primary peptide sequence of LfcinH shows that the overall importance depends on how they are presented in the secondary structure.

Aromatic amino acids greatly influence a peptide's secondary structure, thereby influencing the antiviral activity of the peptides [108, 152]. The importance of individual amino acids in a peptide has been illustrated by describing the peptide sequence with theoretically derived amino acid descriptors derived by Hellberg et al. [167]. These results showed that the terminal amino acids in the Lfcin analogue were of great importance [108]. Although the aromatic nature of these terminal amino acids appears unfavorable, their spatial contribution to the peptide sequence seemed crucial for antiviral activity [153, 167].

A set of short, highly cationic α-helical 21-mer peptides have been made to identify the secondary structural requirements for high anti-HSV activity [152]. The peptides' secondary structures have been described by circular dichrosim (CD) measurements in solutions of liposomes, micelles and a structure-stabilizing buffer [152]. However, the peptides' antiviral activity could not be related to their α-helicity, at least when the secondary structure was described by CD data [152]. This is in agreement with results presented by Strøm et al. [168–169]. They concluded that the flexibility of the peptide and the likelihood of strong interactions with the cell surface make any helicities present in the solution non-existent on the cell surface [168–169].

Both LfcinB with a stable β-sheet structure, some Lfcin analogues with internal disulfide bridges as well as a set of short α-helical peptides possess antiviral activity with presumably similar modes of action [108, 152]. This indicates that the peptides are able to interact with their target, despite large structural differences. Given that all peptides interact with the same target, a possible explanation would be that they adapt an amphipathic conformation. Lfcin is known to fold into an amphipathic shape with hydrophobic residues clustered on one side of the twisted β-sheet, while hydrophilic and positively charged residues are found on the opposite side [149]. A similar amphipathic structure is preserved with a cationic sector in the short α-helical peptides [170]. Precise positioning of charged residues may be crucial for interaction. The spatial positioning of amino acids which contribute to peptide antiviral activity is influenced by other amino acids present in the peptide sequence. This can explain how structural parameters such as hydrophobic and lipophilic amino acids, both in the Lfcin analogues and the short α-helical peptides, contribute to anti-HSV activity [108, 152].

## The cellular target and antiviral mode of action of Lfcin

Similar requirements for spatial presentation of specific residues has been demonstrated for the ability of HSV gC to interact with HS. Trybala et al. [171] have shown that in addition to the charged residues in gC, two hydrophobic residues might be necessary for the specific spatial interaction between the cationic residues and the negatively charged HS. Based on the sequence data for gC of HSV-1 [172], a 16-amino acid loop structure has been described as the functional site involved in binding to cell surface HS [171]. This loop structure has 87.5 % sequence homology with the loop in gC of HSV-2 [173]. Lfcin creates a similar loop of 16 residues [108], with 87.5% homology to gC HSV-1, when comparing structural groups of amino acids, rather than identical amino acids. This may explain the difficulty in creating Lfcin analogues with higher antiviral specificity [108].

Energy-independent cellular uptake of cationic peptides involving cell surface HS has been described [174]. The mechanism is influenced by the arginine content of the peptide [175–177]. Similarly, transmission electron microscopy (TEM) studies have revealed that LfcinB, is able to enter both CS- and HS-deficient cells in an energy-in-

dependent way [55]. This can be explained by a mechanism described by Kim et al., [178] where internalization of arginine-rich peptides is only partially inhibited by heparinase III. The NLS located in LfcinH [130] may also contribute to the peptide shuttle to the nucleus.

HS works as an attachment receptor for HSV. Thus blocking of HS is hypothesized to reduce the viral infection [4–5]. It has been proposed that cationic antimicrobial peptides block HSV infection by binding to HS at the cell surface in a manner similar to Lf [55]. This is supported by the fact that Lfcin has no direct effect on the HSV particle, since allowing Lfcin to interact with the virus did not affect the inhibitory effect of the peptide [55]. Lfcin peptides also contain specific GAG binding domains [109–110] and have shown relatively high HS affinity [108].

The different activity of peptides against HSV-1 and HSV-2 may be attributed to a combination of their amino acid content and their globular structure [101, 108, 152–153, 179]. Similar differences have been reported for polyanionic compounds [180], and may involve the viral specificity for the receptor molecules and/or the peptides' ability to interact with different viral receptors. Trybala et al. [181] have demonstrated that HSV-2 has higher affinity than HSV-1 for HS. These data support the dependence of peptide anti-HSV-2 activity on secondary structure, whereas the anti-HSV-1 activity is more dependent on the net charge in the peptide [108, 152]. HSV-1 has also been shown to be highly dependent on the gC HS interaction [182], while HSV-2 gB plays a key role in mediating HSV-2 attachment and entry [183]. Thus, HS blocking likely affects HSV-1 and HSV-2 interaction differently, explaining why some peptides possess higher activity towards HSV-1 than HSV-2, and vice versa [108, 152].

The 3-O-HS entry receptor is structurally similar to HS, except with an additional sulfate in the 3-OH position of the glucosamine residue. This increase in charge potentially increases the interaction with cationic peptides. Peptide interaction with HS molecules containing 3-O-HS binding sites may interfere or block the HSV gD interaction. This may explain why several peptides have shown higher antiviral activity against HSV-1 than HSV-2 [108, 152], since 3-O-HS serves as an entry receptor for HSV-1 and not for HSV-2 [19].

Antiviral activity observed when LfcinB or short α-helical peptides are added after initial attachment of the virus to the cell surface and after viral entry [55, 152] implies an additional effect of peptides on viral spread from cell to cell. Similar ability to reduce cell-to-cell spread has been reported for α-helical interferon [184]. Although still controversial, it has been suggested that α-defensin-1 is the soluble $CD8^+$ T-cell antiviral factor with potent activity against HIV [185]. Similar immune activating properties are also hypothesized for Lfcin and other cationic peptides.

## Combined drug effect

*In vitro* studies have shown that Lf exhibits synergy in combination with antifungal agents against *Candida* isolates and with zidovudine against HIV-1 [186–187]. A synergistic antiviral activity was also observed for HSV-1 and HSV-2 when ACV was used in combination with Lf or Lfcin [101, 152]. Lf and Lfcin have also been shown to have antiviral activity towards ACV-resistant clinical isolates [101].

## Models for the design of new antiviral peptides

The structural flexibility of peptides has made it difficult to investigate their mode of action and identify their pharmacophore. Theoretically derived amino acid descriptors [167, 188], have been used to minimize this problem and create a mathematical model for predicting a peptide's biological activity. The model has shown that anti-HSV activity, HS/CS affinity, calculated hydrophobicity, net charge and aliphatic index of peptides are well modeled by the amino acid descriptors [153]. Similar models have been used successfully in modeling both antibacterial [189] and anti-cancer activities [190]. The model explains the correlation between a peptide's primary sequence and its biological and chemical activity [108, 153].

## Conclusion

Lf has a high affinity to HS and exhibits high anti-HSV activity when present at the cell surface. Lf is also able to inhibit cell-to-cell spread, and exhibits synergistic antiviral activity in combination with ACV.

Comparably, Lfcin possesses similar anti-HSV activity and high affinity to HS. Interaction with cell surface HS enables Lfcin to block entry of HSV-1. Cell surface localization of Lfcin is not required for antiviral activity, indicating a dual antiviral mode of action. Lfcin exhibits synergy when used in combination with ACV against HSV. The affinity of cationic peptides to HS can explain their antiviral activity. Affinity for HS and anti-HSV activity is dependent on the spatial presentation of charged residues in the peptides. However, peptide secondary structure appears to be of minor relevance to antiviral activity. A mathematical model has proven successful in explaining peptide activity on the basis of peptide sequence.

*Acknowledgement.* Dr. M. D. Brazas is greatly acknowledged for her linguistic assistance.

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