Published in final edited form as:

Curr Opin Virol. 2014 August ; 0: 95–100. doi:10.1016/j.coviro.2014.05.006.

Antiviral lectins as potential HIV microbicides

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Abstract

A growing class of potential antivirals encompasses carbohydrate-binding proteins, such as antibodies and lectins. They block virus entry into host target cells and halt virus transmission from virus-infected cells to non-infected cells, thereby preventing infection. Here, we review the structural basis for the anti-HIV activity of various lectins, describing their structures and determinants of high-affinity oligosaccharide binding. The mechanism of glycan recognition on the gp120 envelope protein by these antiviral lectins may therefore be exploited for developing agents and alternative strategies to prevent HIV transmission.

Introduction

Despite continued and extensive research efforts over the last 30 years, there is still no cure for HIV/AIDS. Therefore, new and different strategies for preventing sexual transmission of HIV are being explored. The development of lectins as microbicidal agents for topical or *ex-vivo* use represents such an alternative approach in the fight against AIDS [1-4]. Topical agents may be particularly useful for curbing the escalating rate of HIV infection in women, notably in those regions of the world where social and psychological barriers to other methods of prevention, diagnosis and treatment of HIV infections may not easily be overcome. The use of microbicides, when applied topically to genital mucosal surfaces, is potentially a powerful strategy to significantly reduce transmission of sexually transmitted viral pathogens to females, given that it is discreet and can be completely controlled by women.

Antiviral lectins prevent infection by binding to the sugars that decorate the surface of the HIV envelope (Env) glycoprotein gp120, keeping the trimeric Env in a closed, nonfusogenic state [1,4-6]. This renders the virus unable to enter the host target cell. It also blocks direct cell-to-cell transmission between virus-infected and non-infected cells [7]. Lectins can also efficiently abrogate DC SIGN-mediated HIV-1 capture and subsequent transfer to T

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lymphocytes [8]. In order to illustrate the molecular basis of their HIV-inactivating properties, we review the atomic structures, the distinct modes of glycan recognition and oligosaccharide binding epitopes of Cyanovirin-N (CV-N), *Oscillatoria agardhii* agglutinin (OAA), Griffithsin (GRFT), Scytovirin (SVN), *Microcystis viridis* lectin (MVL), and Actinohivin (AH).

Antiviral lectins: Similarities and Differences

CV-N, OAA, GRFT, SVN, MVL, and AH exhibit potent anti-HIV activity, with IC₅₀ values in the nanomolar-picomolar range. They were discovered and isolated from a variety of cyanobacterial or algal species. For example, CV-N was found in an aqueous extract from the cyanobacterium *Nostoc ellipsosporum* [7,9], OAA in the *Oscillatoria agardhii* strain NIES-204 [10,11], SVN in *Scytonema varium* [12], and MVL was isolated from the freshwater bloom-forming cyanobacterium *Microcystis viridis* NIES-102 [13]. In addition, GRFT was isolated from the red alga *Griffithsia* sp [14], collected from the waters off New Zealand, and AH from the actinomycete *Longisporum Albid* (actinomycete strain K97-0003) [15,16]. Most importantly, the atomic structures of these lectins have helped to elucidate the basis of their antiviral activity, and their interactions with the relevant high mannose glycans of gp120, revealed either by X-ray crystallography or NMR spectroscopy, yield important details on their distinct modes of glycan recognition, both on the protein and oligosaccharide epitopes.

All the above lectins exhibit different tertiary and quaternary structures. Interestingly, however, they all contain internal repeats within the primary sequences (Figure 1). CV-N, OAA, SVN, and MVL possess two sequence repeats. In CV-N, the two tandem repeats comprise residues 1-50 (sequence repeat 1; SR1) and residues 51-101 (sequence repeat 2; SR2) [9]. Each repeat possesses a disulfide bond, C8-C22 in SR1 and C58-C73 in SR2 (Figure 1A) [9,17,18]. In OAA, residues 1-67 and residues 68-133 make up sequence repeat 1 (SR1) and sequence repeat 2 (SR2), respectively. The OAA repeats exhibit ~80 % sequence identity between SR1 and SR2 (Figure 1B) [11,19]. The SVN sequence also contains sequence duplication for residues 1-48 and residues 49-95 (Figure 1D) [12]. Interestingly, SVN possesses a large number of cysteine residues, ten in total, [12] forming five disulfide bond between C7-C55, C20-C32, C26-C38, C68-C80, and C74-C86 (Figure 1D) [20,21]. The two sequence repeats in MVL each contain 54 amino acids that are ~50% identical (Figure 1F) [13].

GRFT and AH contain three sequence repeats. In GRFT, SR1 comprises residues 1-18 and residues 101-121, SR2 spans residues 19-56, and SR3 contains residues 57-100 (Figure 1C). In addition, distinct sequence motifs were noted in two loop regions, namely GxYxD and GGSGG motifs (Figure 1C). AH's three repeats SR1, SR2, and SR3 encompass residues 1-38, 39-77, and 78-114, respectively (Figure 1E) [16].

Interestingly, the number of sequence repeats often corresponds to the number of domains and binding sites in each lectin, with the exception of GRFT, where the three repeats result in three binding sites, but not three individual domains. However, the polypeptide chain of each sequence repeat does not always make up a domain; frequently individual domains of

these lectins involve strand exchange between domains. The high degree of amino acid sequence similarity, ranging from ~30% in CV-N to ~81% in OAA, goes hand-in-hand with structural similarity between the individual domains.

Overall, each of these lectins has unique features. Some multimerize and/or exhibit domain swapping, while others exist solely as monomer. CV-N and GRFT can domain swap (Figure 2A and 2C), although they can be found as monomers in solution, or can be engineered into a monomer, as was done with GRFT [22]. The nature of the 3D domain swap in CV-N and GRFT is distinctly different; in CV-N, half of the protein's polypeptide chain is involved in the exchange (Figure 2A) while in GRFT only the first two of twelve β -strands domain swap (Figure 2C). MVL, on the other hand, exhibits no domain swapping, but homo-dimerizes (Figure 2F). All of the other lectins discussed here (OAA, SVN, and AH) are monomers (Figure 2B, 2D, and 2E). As to the number of sugar binding sites, they also vary between the different lectins. Monomeric CV-N, OAA, and SVN bind sugars at two sites; AH and the engineered monomeric GRFT bind at three sites; domain-swapped CV-N and MVL exhibit four binding sites; and the lectin with the highest number of binding sites, six in total, is the domain-swapped GRFT.

The most interesting distinction between all these lectins relates to their carbohydrate specificity: each lectin recognizes a different epitope on high mannoses. For example, CV-N binds to the terminal $\text{Man}\alpha(1\rightarrow2)\text{Man}\alpha(1\rightarrow2)\text{Man}$ of the D1 or the terminal $\text{Man}\alpha(1\rightarrow2)\text{Man}$ of the D3 arms in $\text{Man}8/9$ (Figure 2A). Like CV-N, AH also recognizes a $\text{Man}\alpha(1\rightarrow2)\text{Man}$ disaccharide (Figure 2E), however the protein-bound conformation of the sugar is different between these two lectins. In CV-N, both mannoses reside in a shallow binding surface, held in position by nine hydrogen bonds. In contrast, in AH only one of the two mannoses is in close contact with the protein, positioned by a total of four hydrogen bonds. This closer and more specific interaction seen for CV-N may explain the higher potency of CV-N, compared to AH. The glycan specificity of all the other lectins is, again, different. For example, OAA recognizes the mannose core branch of $\text{Man}8/9$ (also called $\alpha2$, $\alpha3$ mannopentaoase core) (Figure 2B); GRFT recognizes any of the terminal mannoses on the D1, D2 or D3 arms of $\text{Man}8/9$ (Figure 2C); SVN binds only to the $\text{Man}\alpha(1\rightarrow2)\text{Man}\alpha(1\rightarrow6)\text{Man}\alpha(1\rightarrow6)\text{Man}$ tetrasaccharide (Figure 2D); and MVL binds to the $\text{Man}\alpha(1\rightarrow6)\text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc}$ tetrasaccharide (Figure 2F).

Interestingly, there appears to exist a correlation between the number of binding sites on the protein and/or the number of epitopes recognized on $\text{Man}8/9$ and a protein's antiviral potency. The most active lectin is domain-swapped GRFT, which inactivates HIV-1 at picomolar concentrations. Both monomeric and domain-swapped CV-N proteins exhibit anti-HIV activity at sub-nanomolar concentrations and are more potent than OAA, SVN, MVL, and AH, which display IC_{50} values in the low to medium nanomolar ranges. Comparing activities in relation to the number of binding sites on the protein and the contacted epitopes on the glycan, it appears that the number of epitopes on the high mannose oligosaccharides that are engaged in the interaction with the lectin is more critical than the number of binding sites on the protein. For example, between CV-N and OAA, both of which possess two binding sites on the protein, CV-N exhibits higher potency than OAA and interacts with two epitopes on the sugar while OAA binds only one sugar epitope. In

line with this observation, CV-N is also more potent than most of the other lectins, such as SVN and MVL, with two and four binding sites, respectively, but only recognizes a single sugar epitope. Note that, both CV-N and AH recognize the same number of epitopes on Man-8/9, with AH containing three binding sites and CV-N only two. However, CV-N is more potent than AH, as the binding affinity of CV-N for the same ligand is tighter than that of AH. Therefore, it is evident that the higher the number of sugar epitopes involved in an interaction and the tighter the protein's binding site interacts with the ligand, the more potent the lectin's activity would be.

Conclusion

The intrinsic antiviral activities of the mannose-binding lectins CV-N, OAA, GRFT, SVN, MVL, and AH render these molecules promising candidates for microbicide development, especially as components in antiviral preparations that can be applied topically. They specifically target the high mannose sugars that decorate the major envelope glycoprotein gp120 of HIV, preventing the necessary conformational change into the active, fusion-competent state. Indeed, preliminary studies with CV-N have been very promising, suggesting that infection with a chimeric SIV/HIV virus in macaques can be curtailed, when delivered by either vaginal or rectal routes [23,24]. However, there are still major hurdles to overcome and safety concerns remain: lectins can induce mitogenic activity in PBMCs, notably in prolonged exposures (>3 days) [25,26]. More studies are necessary, to investigate these shortcomings. Already some initial strategies appear to be effective in reducing mitogenic activity *in vitro*, such as attaching CV-N to polyethylene glycol (PEG) polymer chains [27]. So far, GRFT seems to be devoid of mitogenic activity [28], and no results are available for the other lectins. Given the differences in binding, toxicity, and anti-HIV activity, it may be prudent to include more than one lectin in any potential lectin-based microbicide.

There is still a question as to the most advantageous delivery route for these lectins. A number of modes are being considered. The most straightforward is their formulation in microbicidal preparations for vaginal or rectal application, or possibly, as components in multifunctional contraceptive gels or rings. Another method of delivery, potentially even more promising, may be their *in situ* expression by modified commensal bacteria, especially those that naturally colonize the vagina or gut. This concept has already been explored for CV-N, using the human commensal bacterium *Streptococcus gordonii* [29] or an engineered strain of the natural vaginal *Lactobacillus jensenii* [30]. Therefore, it is likely that the other lectins discussed above will be tested in similar ways. They should be complementary to CV-N, given their differences in sugar epitope specificities, and combinations or hybrid lectins may exhibit increased activity. Further developments of such antiviral lectins as pharmacological agents may yield new and effective ways of interfering with HIV transmission, still an urgent need to prevent suffering and deaths of vulnerable populations worldwide.

Acknowledgments

The authors would like to thank Dr. Teresa Brosenitsch for critical reading of the manuscript. This work was supported by an NIH grant to A.M.G (GM080642).

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Highlights

- Lectins are potential antivirals.
- The structural basis for the anti-HIV activity of various lectins is reviewed.
- The atomic structures of several lectins as well as their sugar binding specificities are described.

Figure 1. Sequence alignment of CV-N (A), OAA (B), GRFT (C), SVN (D), AH (E) and MVL (F) illustrating the sequence repeats. Conserved residues between repeats are highlighted in magenta. Disulfide bonds, alpha helices and beta strands are indicated and colored in yellow, light green and light purple, respectively.

Figure 2.

Structures and carbohydrate specificities of CV-N (A), OAA (B), GRFT (C), SVN (D), AH (E) and MVL (F). The structures are shown in ribbon representation. The chemical structure of Man-9 is also shown, with GlcNAc and Mannose units indicated in the letters G and M, respectively. The three arms of Man-9 are labeled as D1, D2, and D3, respectively. The epitopes recognized by each lectin are shaded either in light magenta or light blue. BS and PM denote binding site and pseudomonomer, respectively.