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Structures of the Complexes of a Potent Anti-HIV Protein Cyanovirin-N and High-mannose Oligosaccharides

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Using x-rays generated at beamline X9B of the National Synchrotron Light Source at Brookhaven National Laboratory, scientists have determined the structure of Cyanovirin-N (CV-N), a protein known for its ability to prevent viral infection. CV-N was complexed to high-mannose oligosaccharides, molecules located on the surface of the virus causing acquired immunodeficiency syndrome (AIDS). CV-N is a promising lead for the design of drugs against AIDS. The molecular structures generated in this study provide atomic details of how CV-N prevents infection by the human immunodeficiency virus (HIV), a virus causing AIDS.

Of the more than 30 million people infected with human immunodeficiency virus (HIV) before 1997, 75 to 85 percent acquired the virus through heterosexual contact, making acquired immunodeficiency syndrome (AIDS) a continuing threat to the general population. Development of a vaccine active against HIV is complicated by the high mutation rate of the virus. So, another way of preventing HIV infection is the development of anti-HIV virucides, which are chemicals that prevent HIV infection. A unique natural product with anti-HIV properties was discovered while screening for new antiviral agents. This protein, originally isolated from cultures of the cyanobacterium (blue-green algae) *Nostoc ellipsosporum*, was named cyanovirin-N (CV-N).

CV-N potently inactivates the two most well known strains of HIV, HIV-1 and HIV-2, as well as their counterparts in monkeys, the simian immunodeficiency virus (SIV), and cats, the feline immu-

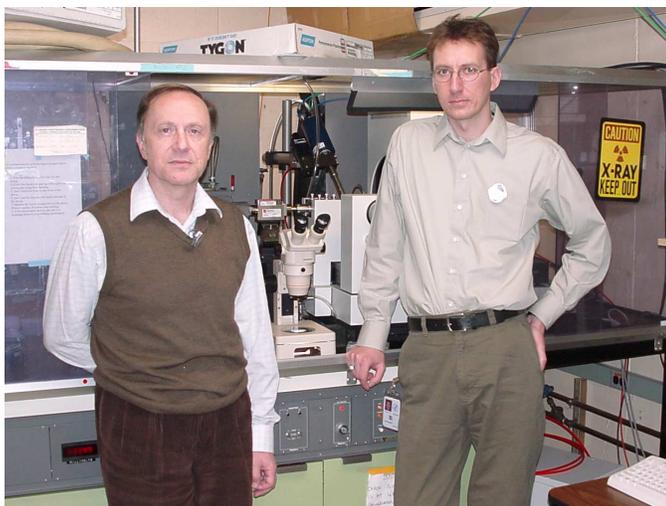
nodeficiency virus (FIV). CV-N prevents HIV-1 from infecting host cells by interfering in key interactions between the glycoprotein gp120, which is present on the HIV-1 envelope, and receptors on cells that are about to be infected by HIV-1. Understanding the structural basis of such interactions is important for the potential development of CV-N as an anti-AIDS drug.

CV-N, a 101-amino acid protein, can exist in solution either as a monomer or a dimer. When in the form of a monomer, the protein

consists of two similar domains with an overall ellipsoidal shape. Each domain contains mostly β -strands and loops (figure 1-A). A change of torsion angles in the central, or hinge, region between the two domains separates them into an extended form, in which they do not interact with each other (figure 1-B).

The CV-N dimer is formed by two extended monomers that swap their domains. By naming A and B the domains of the first monomer, and A' and B' those of the second monomer, the overall structure of CV-N is made of the combinations AB' and A'B, called pseudomonomers, which are linked to each other through a hinge region.

We investigated how the monomeric and dimeric forms of CV-N bind to two branched oligosaccharides (organic compounds that include sugars, starches, celluloses, and gums): oligomannose-9 (Man-9) and a synthetic hexamannoside, which have a high and low affinity to CV-N, respec-



Alexander Wlodawer (left) and Istvan Botos.

tively. Man-9 was derived from natural sources and its structure corresponds to that of glycoprotein gp120, the chemical present on the HIV-1 envelope. The synthetic hexamannoside has a similar core mannose structure to Man-9.

A CV-N monomer can bind to oligosaccharides on two distinct sites: a high affinity, primary site, and a low affinity, secondary site (figure 2). We have shown that the binding sites exhibit different affinities for the oligosaccharides. The CV-N domain-swapped dimer exhibits four sugar-binding sites: two primary sites near the hinge region, and two secondary sites on the

opposite sides of the dimer.

In both monomeric and dimeric forms of CV-N, the primary sugar-binding site consists of a deep pocket in the close proximity of the hinge region. We have shown that the shape of this site is directly influenced by the hinge and relative orientation of the domains.

The secondary sugar-binding site, unaffected by the hinge region, has the same conformation in both the monomeric and dimeric CV-N. The molecular structures of CV-N bound to Man-9 and a synthetic hexamannoside show that the binding interface is formed by three

mannose rings in the case of Man-9 and two in the case of the hexamannoside. So, the additional binding affinity of CV-N for Man-9, as compared to the hexamannoside, results from the additional binding energy of the third mannose ring's interaction with CV-N.

The ability of CV-N to target virus-associated oligosaccharides with high affinity, while binding mammalian oligosaccharides, such as Man-6, with comparably low affinity, is the basis for the potential use of CV-N to inhibit HIV infection.

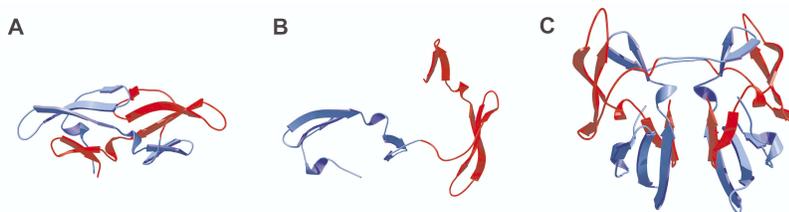


Figure 1. The monomeric form of cyanovirin-N (CV-N) (A) has two similar domains, A (red) and B (blue), linked by a hinge region. A change in torsion angles in the hinge region separates the domains into an extended form (B). A domain-swapped dimer (C) is formed by two such extended monomers. Each pseudo-monomer (AB' and A'B) is virtually identical to the compact monomer with the exception of the hinge residues.

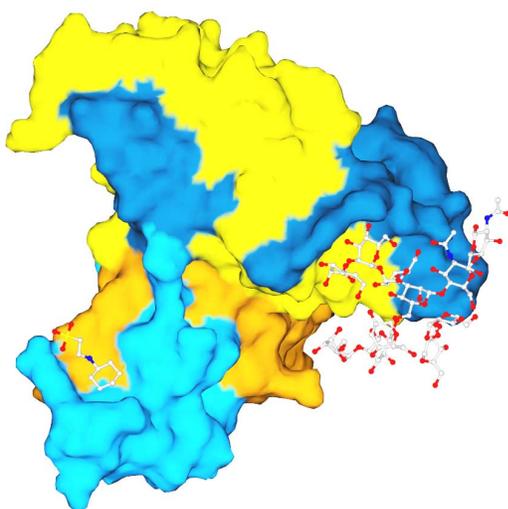


Figure 2. Crystal structure of the domain-swapped cyanovirin-N dimer. The molecular surface shows the primary and secondary oligosaccharide-binding sites, with a 2-(Cyclohexylamino)ethanesulfonic acid (CHES) molecule bound to primary site (left) and oligomannose-9 to the secondary site (right). Domains A and B of the first molecule are shown in dark and light blue, respectively, and domains A' and B' of the second molecule are shown in orange and yellow, respectively.

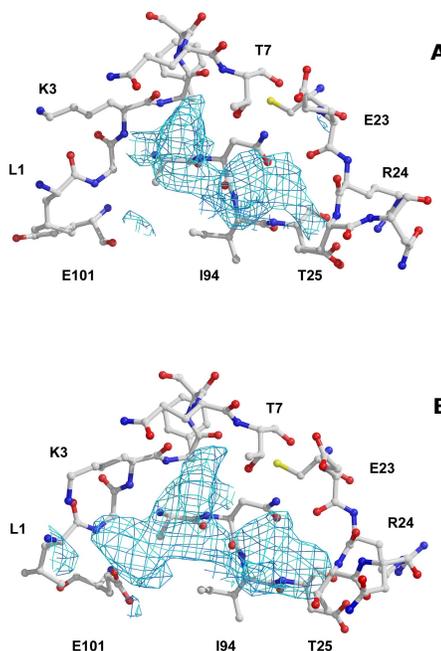


Figure 3. Sugar-binding areas on the surface of cyanovirin-N and the electron density maps of bound carbohydrate ligands: (A) cyanovirin-N and hexamannoside, and (B) cyanovirin-N and oligomannose-9. The binding interface is formed by three mannose rings in the case of oligomannose-9 and by two rings in the case of the hexamannoside, thus explaining the higher binding affinity of cyanovirin-N for oligomannose-9 than for the hexamannoside.