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ACCELERATED COMMUNICATION

Cyanovirin-N Defines a New Class of Antiviral Agent Targeting N-Linked, High-Mannose Glycans in an Oligosaccharide-Specific Manner

ANDERS J. BOLMSTEDT, BARRY R. O'KEEFE, SHILPA R. SHENOY, JAMES B. MCMAHON, and MICHAEL R. BOYD

Department of Clinical Virology, University of Göteborg, Sweden (A.J.B.) and Laboratory of Drug Discovery Research and Development, Division of Basic Sciences, National Cancer Institute, Frederick, Maryland (B.R.O., S.R.S., J.B.M., M.R.B.)

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ABSTRACT

Herein we report that the novel HIV-inactivating protein cyanovirin-N (CV-N) targets specific, N-linked high-mannose oligosaccharides found on the viral envelope of HIV-1. First, we released the oligosaccharides by PnGase-treatment of HIV-gp120 (containing high-mannose, hybrid-type and complex-type oligosaccharides) or HSV-1 gC (containing only complex-type). Then, in an affinity chromatographic system, we found that CV-N bound to the free oligosaccharides from gp120 but not from gC-1, suggesting that high-mannose oligosaccharides constitute a target structure for CV-N. This was supported by the affinity of CV-N for high-mannose glycans released from gp120 by endo-H as well as high-mannose glycans released from castanospermine-treated HSV-1 gC. Furthermore, free Man-8 or Man-9 oligosaccharides partially inhibited the binding of CV-N to gp120, although neither oligosaccharides smaller than Man-7 nor monosaccharides interfered with CV-N/gp120 interaction, thereby establishing the oligosaccharide-specific affinity of CV-N to high-mannose glycans. This affinity for high-mannose oligosaccharides may explain the broad antiviral activity of CV-N against human and primate immunodeficiency retroviruses as well as certain other viruses that carry these oligosaccharides.

Cyanovirin-N (CV-N), an 11-kDa protein originally purified from extracts of the cultured cyanobacterium, *Nostoc ellipsosporum*, potently inactivates a broad spectrum of HIV strains including T-tropic, M-tropic, dual-tropic, and primary HIV-1 isolates and related immunodeficiency viruses (Boyd et al., 1997). CV-N comprises a unique sequence of 101 amino acids (Gustafson et al., 1997), and its tertiary structure, elongated and largely β-sheet with internal 2-fold pseudo-symmetry, is unprecedented (Bewley et al., 1998). CV-N has been produced recombinantly in *Escherichia coli* and the recombinant version was shown to be identical to native CV-N (Mori et al., 1998). Given its lack of toxicity and high resistance to physicochemical denaturation, CV-N is a promising new anti-HIV substance both for potential therapeutic and prophylactic applications (Boyd et al., 1997; Esser et al., 1999; Dey et al., 2000).

CV-N binds to viral envelope glycoproteins and interferes with viral interactions with target cell receptors essential for viral entry and cell-to-cell fusion; however, a specific structural basis for glycoprotein binding and anti-HIV activity of CV-N has not been fully defined (Boyd et al., 1997; Esser et al., 1999, O'Keefe et al., 2000). Previous studies have revealed that CV-N binds with high affinity to the HIV-1 surface glycoprotein gp 120 (Boyd et al., 1997), however, it does not bind significantly to nonglycosylated gp120 expressed in bacterial cells (Boyd et al., 1997). These observations, together with recent data showing binding of CV-N to a glycosylated form of the transmembrane glycoprotein gp41, but not to nonglycosylated gp41 (O'Keefe et al., 2000), suggest

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ABBREVIATIONS: CV-N, cyanovirin-N; gp, glycoprotein; HM, high mannose; HT, hybrid type; CT, complex type; RT, room temperature; GlcN, glucosamine hydrochloride; Endo H, endoglycosidase H; Con A; concanavalin A; HSV, herpes simplex virus; GMK, green monkey kidney; Man-, oligomannose-; FITC, fluorescein isothiocyanate.

that the carbohydrate components of HIV envelope glycoproteins may be important for the binding and antiviral activity of CV-N.

HIV-1 gp120 is one of the most highly glycosylated viral proteins known, with a carbohydrate component constituting approximately 50% of the total molecular weight (Geyer et al.,1988). The carbohydrate moiety of HIV-gp120 consists of 24 N-linked oligosaccharides, 11 of which are believed to be of high-mannose (HM) or hybrid type (HT) and 13 of complex type (CT) (Fig. 1) (Leonard et al., 1990). In this study, we have characterized the CV-N/glycoprotein interactions by analyzing the binding of CV-N to free, N-linked oligosaccharides released not only from HIV-1 gp120 but also from other glycosylated proteins. We have also analyzed the effect of removing N-glycans from glycoproteins and preincubating CV-N with specific high-mannose oligosaccharides on the CV-N/glycoprotein interactions.

Materials and Methods

Enzyme-Linked Immunosorbent Assay. Recombinant HIV-1_{IIIB} soluble gp120 (sgp120) at a concentration of 1 μg/ml in 100-μl aliquots of PBS was bound to individual wells of 96-well protein-binding assay plates (Immobilon; Nunc, Naperville, CT) by incubation for 2 h at RT. Thereafter, the plates were washed three times with TPBS (PBS augmented with 0.05% Tween 20), then blocked by the addition of 200 μl/well of a solution of 1% bovine serum albumin (Sigma, St. Louis, MO) in PBS, followed by overnight incubation at 4°C and a further wash with TPBS. CV-N (100 ng/well) was then allowed to incubate for 1 h at RT in either PBS or 0.5 M concentrations of the following monosaccharides: fucose, xylose, glucose, galactose, mannose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and sialic acid before addition to the gp120-bound plate. The CV-N samples were then added to triplicate wells and allowed to incubate for 1 h, followed by a TPBS wash, and then by addition and 1 h incubation with 100 μl of a 1:1000 dilution of rabbit-anti-CV-N polyclonal antibodies in each well. Subsequently, 100 μl of a 1:1000 dilution of goat-anti-rabbit secondary antibodies ligated to alkaline phosphatase (Boehringer Mannheim, Indianapolis, IN) was added to each well and allowed to incubate for 1 h at RT. This incubation was followed by a TPBS wash and incubation with alkaline phosphatase substrate buffer (10% diethanolamine, 4 mg/ml *p*-nitrophenylphosphate, 1 mM $MgCl_2$, pH 9.2). Absorbance was measured at 405 nm. CV-N used in all studies was produced recombinantly in *E. coli* as described previously (Mori et al., 1998).

Metabolic Labeling of HIV-gp120 and HSV-gC Carbohydrates. The radiolabeling of HIV-gp120 oligosaccharides was performed according to Hemming et al. (1996). Briefly, 6×10^6 H9 cells were infected with HIV-1_{LAV} and cultivated for 24 h in 5 ml of culture medium (RPMI 1640, 20% fetal bovine serum, 2 μM glutamine, 100 IU/ml penicillin, and 100 IU/ml streptomycin). Cultures were spun and cells resuspended in 6 ml of growth medium containing 300 μCi of D-[^{3}H]glucosamine hydrochloride ([^{3}H]GlcN; specific activity, 30 Ci/mmol; Amersham Pharmacia Biotech, Piscataway, NJ) and cultivated for 72 h. Cells were washed in PBS and sonicated in 0.5 ml of lysis buffer (1% triton X-100, 1 mM EDTA, 100 mM NaCl, 20 mM Tris-HCl, pH 8.0, and 100 IU aprotinin). Radiolabeling of HIV-1 gp120 and HSV-1 gC expressed in GMK-cells was performed as described previously (Bolmstedt et al., 1991; Olofsson et al., 1999).

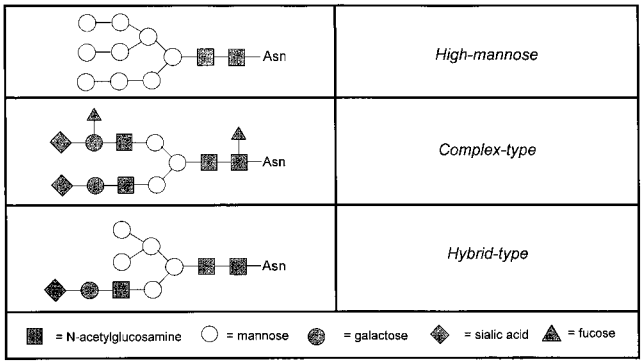

Fig. 1. Subgroups of N-linked glycans.

Affinity Chromatography of Radiolabeled Oligosaccharides. Radiolabeled HIV-gp120 or HSV-gC was immunoprecipitated using monoclonal antibodies (F58/H3 and B1C1B4, respectively), and subjected to *N*-glycosidase (Roche Molecular Biochemicals, Mannheim, Germany) treatment as described previously (Olofsson et al., 1999). The samples were then passed through C18 columns (Waters, Milford, MA) according to the manufacturer's instructions and stored at −20°C until affinity chromatography. Rabbit anti-CV-N polyclonal antibodies (Boyd et al., 1997), were coupled to Protein-A-Sepharose (CL-4B) (Amersham Pharmacia Biotech) in micro columns (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. Thereafter, the columns were washed with TBS-T20 [Tris-buffered saline containing 0.5% (v/v) Tween 20], then 0.4 μM CV-N (2×50 μl) was added at 5-min intervals to one of the columns. The corresponding volume of TBS was added to the "mock" column. After adsorbence at RT for 1 h, the columns were washed as above with TBS-T20. Radiolabeled oligosaccharide samples were loaded in aliquots of 100 μl to the columns at 5-min intervals. After the final sample fraction was loaded, the samples were adsorbed for 30 min at RT. The columns were then washed with TBS-T20 until the radioactivity in the eluate reached background (i.e., basal levels of radioactivity). Fraction volumes of 0.5 ml were collected and analyzed in a beta-counter (Beckman, Palo Alto, CA). Bound material in the columns was eluted from the Sepharose using 0.1 M glycin-HCL pH 2.5, and elution fractions were analyzed for radioactivity in a beta-counter as described above.

Endoglycosidase H Deglycosylation of gp120 and RNase B. Either HIV-1_{IIIB} sgp120 (200 μg; ABI, Columbia, MD) or RNase B (1 mg; Sigma, St. Louis, MO) was taken up in 0.4 ml of 0.5 M NaCl, 100 mM sodium acetate buffer, pH 5.7. To these solutions, either 100 mU (gp120) or 20 mU (RNase B) of endoglycosidase H (Endo H; Glyko Inc., Novato, CA) was added in 20 μl and the reaction mixture was incubated overnight at 37°C. After deglycosylation, the reaction mixtures were centrifuged on a 3-kDa ultrafiltration membrane (Amicon, Beverly, MA) to separate the deglycosylated glycoproteins from released oligosaccharides.

Fluorescence Polarization. Fluorescence polarization studies were performed on a FPM-1 fluorescence polarization detector (Jolley Consulting and Research Inc., Grayslake, IL). Each experiment was initiated by the addition of BODIPY-CV-N to fluorescence polarization buffer (Pan Vera, Madison, WI) resulting in a final concentration of 13 nM BODIPY-CV-N. After transfer of 1 ml of this solution to individual tubes, measurement of fluorescence polarization at 30-s intervals in the presence or absence of added gp120 (either 18 nM recombinant HIV-1_{IIIB} sgp120 or Endo H-deglycosylated recombinant HIV-1_{IIIB} sgp120) was performed. For the HM oligosaccharide studies, 10 μg of either Man-5, Man-6, Man-7, Man-8, or Man-9 (Glyko Inc., Novato, CA) was added to the BODIPY-CV-N before the addition of gp120. The manufacturer determined the quantity of the individual oligosaccharides.

Viral Inactivation by CV-N or Con A. The anti-HIV activity of CV-N was monitored essentially as described previously (Boyd et al., 1997) using the CD4-positive cell line MT-2 (Haertle et al., 1988) maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 IU penicillin per ml, 20 μg/ml gentamicin, and 100 IU/ml streptomycin. Briefly, CV-N or concanavalin A (Con A) were incubated at 37°C for 90 min in presence or absence of 10% α-methylmannoside (Sigma). Thereafter, cell-free virus was added and incubated for 1 h at 37°C. Virions were pelleted by ultracentrif-

ugation and resuspended in culture medium and added to cells. The infected cultures were incubated at 37°C, and virus antigen (p24) in culture supernatant was measured 4 to 6 days after infection, as described by Vahlne et al. (1991).

Results

Previous studies on the binding of CV-N to HIV-1 gp120 have suggested that the CV-N/gp120-interaction is carbohydrate-dependent (Boyd et al., 1997; Esser et al., 1999; O'Keefe et al., 2000). To test directly whether CV-N binds to the carbohydrate component of HIV-gp120, we analyzed the CV-N binding to free N-linked glycans released from gp120 by PnGase treatment. This treatment detaches all N-linked glycans from the peptide by converting Asn to Asp (Tarentino et al., 1985). The radiolabeled carbohydrate fractions were then analyzed for CV-N binding by affinity chromatography using Protein A-Sepharose columns with or without CV-N coupled by anti-CV-N antibodies. In this chromatographic system, we found that radiolabeled carbohydrates were captured by the column containing the Sepharose-anchored CV-N but not by the corresponding column lacking CV-N, indicating that CV-N binds to N-linked oligosaccharides on gp120 (Fig. 2). This is the first experimental result indicating that CV-N can bind sugar-based structures lacking any proteinaceous component.

To determine whether it was possible to inhibit the CV-N/gp120 binding interaction by the presence of high concentrations of monosaccharides, we used an enzyme-linked immunosorbent assay system to monitor the capture of CV-N by gp120 attached to a solid support. Purified gp120 was coated onto 96-well plates and CV-N was allowed to bind to gp120 after preincubation in the presence of a ~10,000-fold molar excess of individual monosaccharides including mannose, glucose, galactose, xylose, fucose, sialic acid, *N*-acetyl glucosamine, and *N*-acetyl galactosamine. There was no significant reduction in CV-N/gp120 binding by the presence of any of these monosaccharides (data not shown) suggesting that a higher-order glycosidic structure, rather than a particular terminal monosaccharide unit on gp120, might be the binding target for CV-N.

To determine which types of oligosaccharide structures (HM, HT, or CT) (Fig. 1) were preferentially recognized by CV-N, we first analyzed the CV-N binding to free CT oligosaccharides (Fig. 3). These oligosaccharides were labeled with [^{3}H]GlcN and released by PnGase treatment of the glycoprotein C specified by herpes simplex virus type 1 (HSV-1), grown in green monkey kidney (GMK) cells (Günalp, 1965). Under these experimental conditions, the HSV gC-1 surface glycoprotein is known to contain only CT N-linked oligosaccharides (Wenske et al., 1982; Olofsson et al., 1999). Using the same affinity chromatography system as above, we found no binding of radiolabeled CT oligosaccharides to the CV-N affinity column, thereby indicating that CV-N was not able to bind to CT N-glycans (Fig. 3A). This result correlates well with recently published data on CV-N binding to HIV envelope glycoproteins that indicated that N-linked CT oligosaccharides might not participate in CV-N/glycoprotein interactions (O'Keefe et al., 2000)

In contrast, treating purified [^{3}H]GlcN-labeled gp120 (expressed in the same GMK-cell system as the HSV-1 gC) with Endo H resulted in the specific release of HM and HT oligosaccharides (Trimble et al., 1987). In this instance, the resulting free oligosaccharides bound significantly to the CV-N affinity column but not to the control column (Fig. 3B). Taken together, the observed CV-N affinity for N-linked HM/HT oligosaccharides and lack of affinity for CT oligosaccharides suggests that CV-N binding to viral envelope glycoproteins is largely mediated by interactions with HM/HT oligosaccharides.

To confirm that CV-N binds to unprocessed, N-linked oligosaccharides, we analyzed the binding activity of CV-N to N-linked oligosaccharides released from HSV-1 gC produced in presence of a glucosidase I inhibitor (castanospermine). This inhibitor arrests the N-glycans at a high mannose level before they can be further processed into the HT and CT structures (Datema et al., 1987). We found that these N-linked oligosaccharides likewise bound to the CV-N affinity column (Fig. 3C). As expected, oligosaccharides released from gp120 produced in the presence of castanospermine also bound to CV-N (data not shown). These data confirm that CV-N binds to unprocessed, N-linked oligosaccharides and show that CV-N binding to carbohydrates from HSV-1 gC could be induced by converting the glycans from CT into the HM type. Furthermore, the results also indicate that the CV-N/carbohydrate interaction was independent of the origin of the peptide backbone of the target glycoprotein.

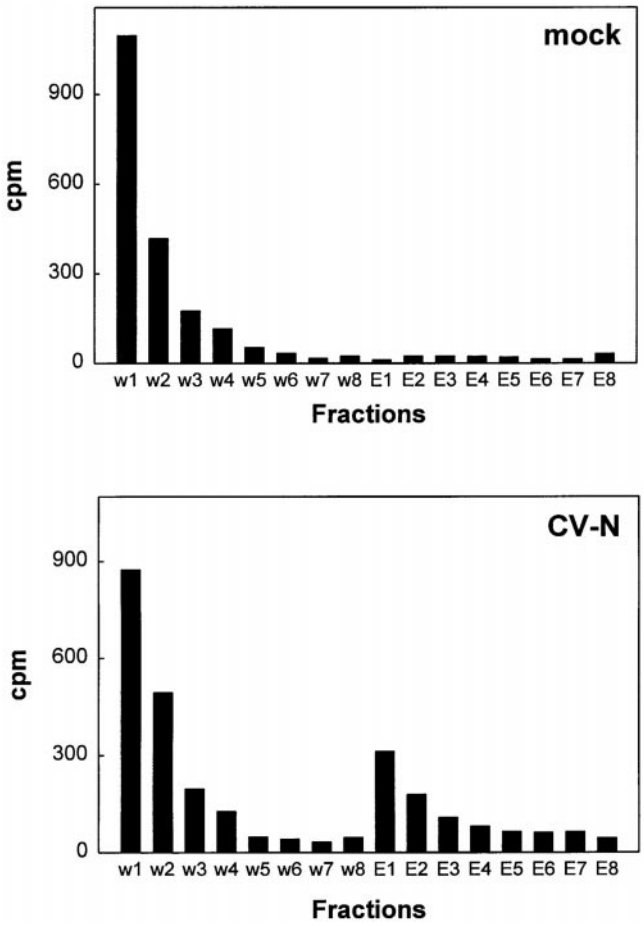

Fig. 2. Binding of CV-N to free oligosaccharides released from HIV-gp120. ^{3}H-labeled oligosaccharides released from gp120 by PnGase treatment were analyzed for binding to CV-N in an affinity chromatography assay. Mock, column containing Sepharose and coupling antibody only; CV-N, column containing Sepharose-coupled CV-N; w, wash fraction; E, elute fraction.

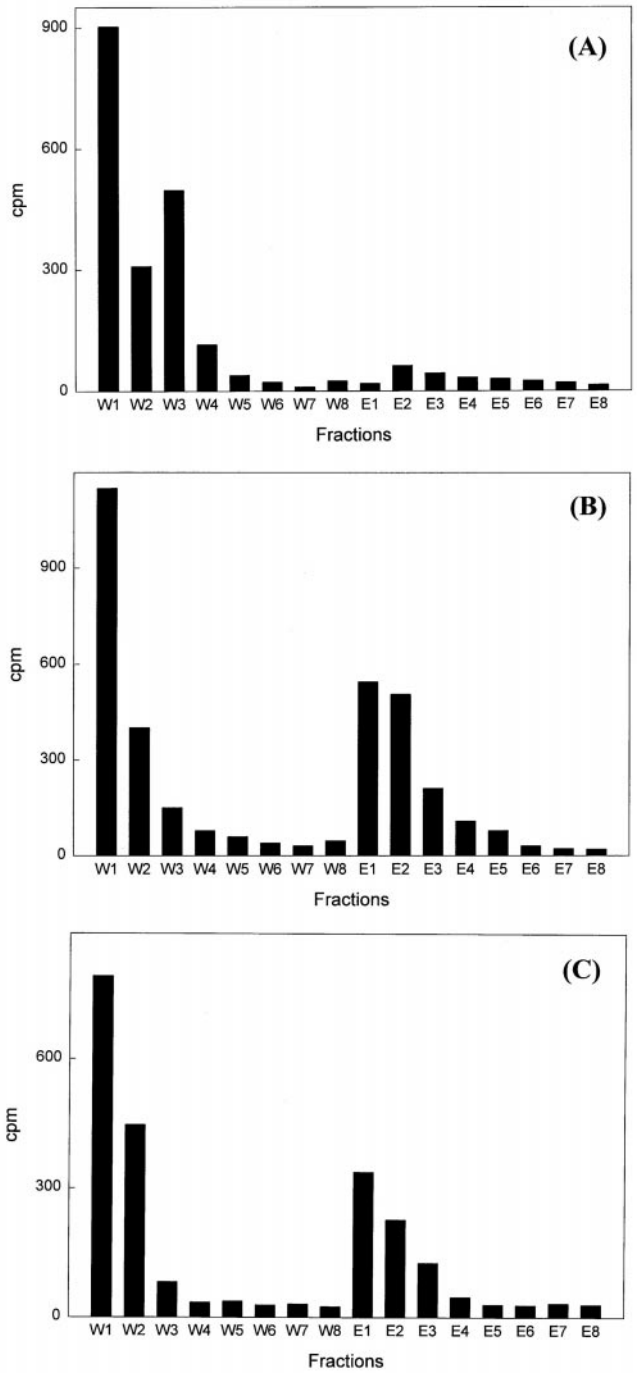

Fig. 3. Binding capacity of CV-N to oligosaccharides. A, ^{3}H-labeled, N-linked complex-oligosaccharides released from HSV-1 gC by PnGase treatment; B, ^{3}H-labeled, N-linked high-mannose/hybrid-type oligosaccharides released from gp120 by Endo H treatment; and C, ^{3}H-labeled, N-linked oligosaccharides released from castanospermine-treated HSV-1 gC by PnGase digestion were analyzed for binding to CV-N coupled to Sepharose in an affinity chromatography assay as described above. w, wash fraction; E, elute fraction.

To characterize the structural preference of CV-N for high mannose oligosaccharides, we analyzed the capacity of a panel of free high mannose oligosaccharides, [i.e., oligomannose-5, -6, -7, -8 or -9 (Man-5, -6, -7, -8 or -9)] to block the CV-N/gp120 interaction in a fluorescence polarization assay previously used to evaluate CV-N/gp120 binding (O'Keefe et al., 2000). We found that Man-8 reduced the CV-N/gp120 binding by almost 50% and Man-9 by 20% whereas oligosaccharides of lower mannose number (i.e., Man-5, -6, and -7) caused no inhibition (Table 1). These data indicate that CV-N binds to N-linked Man-8 and -9 HM-oligosaccharides, but not to further processed HM-glycans (i.e., Man-7 or lower).

The hypothesis that HM oligosaccharides are a target for CV-N binding to glycoproteins was further tested by examination of the binding of CV-N to bovine RNase B, which is known to contain a single N-linked oligosaccharide of exclusively the HM type (Williams and Lennarz, 1984). Using the same fluorescence polarization assay, the results clearly showed significant binding of FITC-CV-N to fully-glycosylated RNase B (Fig. 4A). Furthermore, the CV-N binding to RNase B was essentially eliminated by Endo H treatment, which removed all attached HM oligosaccharides from the glycoprotein (Fig. 4A). These results further support the hypothesis that the binding of CV-N to proteins is determined by its interaction with HM oligosaccharides.

To confirm that the HM oligosaccharides on gp120 were also crucial to the association between CV-N and gp120, we used the fluorescence polarization system to measure the interaction between either fully glycosylated or Endo H-deglycosylated HIV-1 gp120 and FITC-labeled CV-N. The deglycosylation of gp120 by Endo H was not complete but did result in a reduction in the molecular mass of gp120 from 120 kDa to approximately 85 kDa (as determined by SDS-polyacrylamide gel electrophoresis, data not shown). In the ensuing fluorescence polarization study, the deglycosylation of gp120 by Endo H resulted in a decrease in fluorescence polarization of approximately 75% compared with the fully glycosylated protein (Fig. 4B). This indicates that the association between CV-N and the Endo H-sensitive oligosaccharides on gp120 is largely responsible for the interaction between the two proteins.

To test whether mannose monosaccharide units were able to decrease the HIV inactivation capacity of CV-N against whole virus in an in vitro system, we preincubated CV-N with or without high concentrations of α-methylmannoside before adding the CV-N to virions. As a control, we used Con A, a mannose-binding lectin known to inactivate HIV through its interaction with gp120 (Gram et al., 1994) (Table 2). We found that the HIV-inactivation by Con A was diminished dramatically by its pretreatment with α-methylmannoside. In contrast, no significant difference in HIV inactivation was observed between untreated or mannose-treated CV-N. These results further confirmed the unique oligosaccharide-specific targeting of CV-N in HIV inactivation.

Discussion

Our results indicate that cyanovirin-N represents a unique class of antimicrobial agent that specifically targets N-linked glycans of the HM type in an oligosaccharide-dependent

TABLE 1
Inhibition of gp120 binding to CV-N by free oligomannose glycans in flourescence polarization assay

Oligomannose	% Inhibition
Man-5	
Man-6	
Man-7	
Man-8	45
Man-9	20

manner. CV-N had previously been suggested to interact with the carbohydrate component of HIV-gp120, based on the observed binding of CV-N to glycosylated gp120, but not to unglycosylated gp120 (Boyd et al., 1997). Herein we demonstrate an active binding of CV-N to free oligosaccharides enzymatically released from the gp120 peptide backbone, directly implicating a CV-N/carbohydrate-interaction mechanism for the anti-HIV activity of CV-N.

Furthermore, CV-N exhibits unique structural requirements for carbohydrate interaction. Thus, in a chromatography system where free, N-linked oligosaccharides were allowed to bind to CV-N in the absence of the polypeptide components of the glycoprotein, we found that the CV-N bound to N-linked HM oligosaccharides but not to CT oligosaccharides. The importance of HM oligosaccharides for CV-N/glycoprotein interactions was confirmed by the significant loss of CV-N binding to glycoproteins depleted of HM glycans by Endo H treatment, releasing HM and some HT sugars but not CT glycans (Trimble et al., 1987). Important to note is that by analyzing the ability of free oligosaccharides to block the CV-N/gp120-binding, we demonstrate that only Man-8 or -9, but not further processed HM-oligosaccharides, (i.e., Man-7 or lower) could interfere with this interaction. Together, these data emphasize a unique high-order HM-oligosaccharide specificity of CV-N. Our data do not completely rule out a possible additional CV-N affinity for N-linked HT oligosaccharides, a possibility that is currently under investigation.

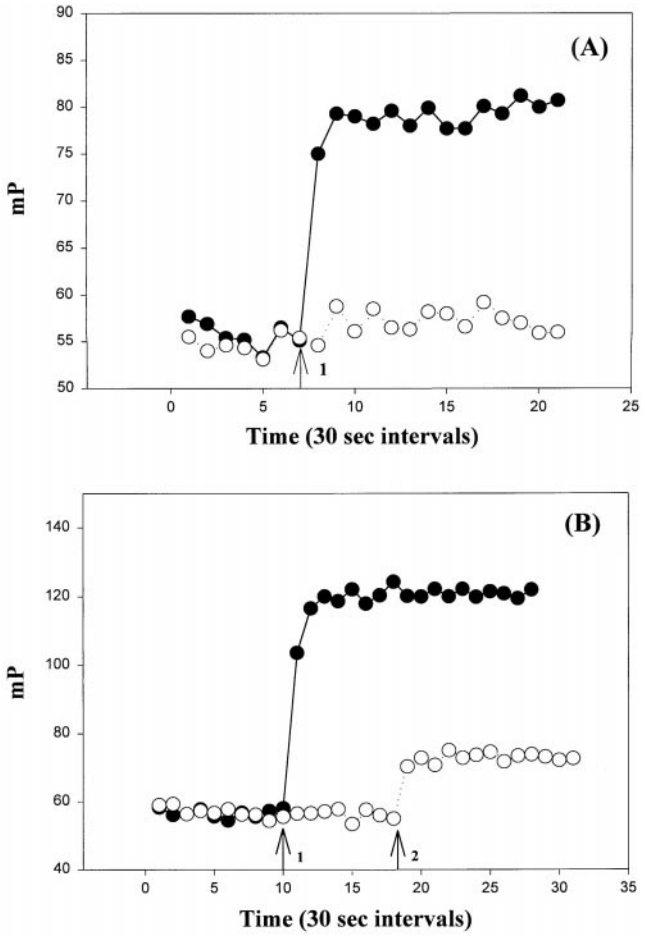

Fig. 4. Binding of FITC-CV-N to Endo H-treated or untreated RNase B and gp120. The binding of FITC-CV-N to either control ● or Endo H-treated ○ RNase B (A) and control ● or Endo H-treated ○ gp120 (B) was detected by a change in fluorescence polarization. Arrows indicate the point at which fully glycosylated or Endo H-deglycosylated glycoproteins were added to FITC-CV-N.

Interestingly, in contrast to classical carbohydrate-interactive lectins such as concanavalin A, wheat-germ agglutinin, jacalin, and *Galanthus nivalis* agglutinin, which have been reported to have anti-HIV activity presumably mediated through interactions with gp120 (Favero et al., 1993; Gilljam, 1993; Gram et al., 1994; Olofsson et al., 1999), the CV-N/gp120-interaction is not inhibited by presence of high concentrations (e.g., 10,000-fold excess) of monosaccharides. This important distinction between CV-N and the lectins suggests that, unlike the latter, which associate with gp120 in a monosaccharide-specific manner and are therefore inhibited by addition of exogenous monosaccharides, CV-N/gp120 interactions are defined by a more complex oligosaccharide-specific binding phenomenon. Taken together, the results described here suggest that 1) CV-N binds to N-linked glycans in the absence of any glycoprotein polypeptide, increasing the potential breadth of CV-N activity against other pathogens than HIV (see below); and 2) CV-N is unique in that it exhibits unusual structural requirements for HM oligosaccharides, mediating binding to N-linked glycans.

The exact nature of the unique carbohydrate recognition of CV-N is yet to be elucidated, but, based on our data, one could speculate that the target structure must encompass the terminal portion of Man-8 and -9 glycans because the core structure of all HM oligosaccharides are identical. Further understanding of the novel oligosaccharide-dependent carbohydrate recognition of CV-N will be important for the potential use of CV-N as an antimicrobial agent as well as a tool in glycobiological studies.

Our data showing that CV-N binds to the abundant HM oligosaccharide structures on gp120 may explain the recently reported interference of CV-N with the gp120-binding of the monoclonal antibody 2G12, which recognizes an HM-rich region on gp120 (Esser et al., 1999). The latter report showed that binding of CV-N to gp120 inhibited the subsequent binding of 2G12 to gp120 but not vice versa, (i.e., prior binding of 2G12 did not block binding of CV-N) (Esser et al., 1999). If CV-N were allowed to bind to the HM glycans on gp120 before addition of 2G12, it is likely that CV-N molecules would partly cover the 2G12 epitope, thereby blocking the binding of the monoclonal antibody. In the opposite situation, in which 2G12 is binding gp120 before CV-N, some HM glycans might be masked by the 2G12 molecule but so many HM glycans in other parts of the gp120 molecule are still accessible for CV-N binding that no significant decrease in CV-N binding to gp120 might be observed in that assay system. This conclusion correlates well with recent publications on the apparent stoichiometry of the CV-N/gp120 bind-

TABLE 2
Mean EC_{50} values for Con A vs. CV-N pretreated with or without (mock) mannose monosaccharide units before addition to HIV-1_{BRU}

Agent	Pretreatment	
	Mock	Mannose
Con A (nM)	20	>300
CV-N (nM)	0.9	0.8

ing interaction which show that CV-N binds multiply to HIV-gp120 (O'Keefe et al., 2000).

CV-N is a promising new candidate for anti-HIV therapeutics and prevention because of its broad antiviral spectrum and its physical stability. In addition, no CV-N-resistant HIV-variants have been identified even after prolonged exposures to CV-N (J. B. McMahon and M. R. Boyd, unpublished observations; A. J. Bolmstedt et al., unpublished observations). The data presented here showing that CV-N binds HM N-linked oligosaccharides may explain the extremely broad antiviral activity against immunodeficiency retroviruses despite the fact that these viruses exhibit extremely high amino acid variation in their envelope glycoproteins. Furthermore, the affinity of CV-N for HM oligosaccharides can also explain recent data on the sensitivity of some other enveloped viruses to CV-N. Specifically, those viruses so far reported sensitive to CV-N (i.e., HIV, feline immunodeficiency virus, simian immunodeficiency virus, measles, and human herpes virus-6) (Boyd et al., 1997; Dey et al., 2000) expose abundant HM oligosaccharides on their surface glycoproteins. In contrast, those viruses demonstrated to be resistant to CV-N (i.e., HSV-1, CMV) expose a higher number of more completely processed carbohydrate side chains on their surface glycoproteins (Wenske et al., 1982; Benko and Gibson, 1986; Boyd et al., 1997; Dey et al., 2000).

In conclusion, CV-N represents a new class of high-order oligosaccharide-specific agent that shows unique carbohydrate-recognition requirements for oligosaccharide structures that are rare in normal human tissue (Ezekowitz and Stahl, 1988). As such, CV-N establishes the potential for oligosaccharide-specific therapeutics with a range of potential activities against not only viruses but possibly also certain bacteria and other pathogens.

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Send reprint requests to: Dr. Michael R. Boyd, Laboratory of Drug Discovery Research and Development, Division of Basic Sciences, National Cancer Institute-Frederick, Bldg. 1052, Rm. 121, Frederick, MD 21702-1201. E-mail: boyd@dtpax2.ncifcrf.gov