Anti-HIV Activity and Conformational Studies of Peptides Derived from the C-Terminal Sequence of SDF-1

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Received October 20, 2003

The entry of the human immunodeficiency virus type 1 (HIV-1) into target cells requires the interaction of viral envelope glycoprotein, gp120, with the human CD4 glycoprotein and a chemokine receptor, usually CCR5 or CXCR4. The natural ligand for CXCR4 is the chemokine SDF-1 that inhibits entry and replication of X4 HIV-1 strains. SDF-1 is produced in two forms, SDF-1α (68 residues) and SDF-1*β* (72 residues); the difference between them lies in the additional four C-terminal amino acids in the SDF-1*β* sequence. Despite the relevance of the N-terminal site in determining the SDF anti HIV-1 activity, SDF-1*β* has a stronger activity than SDF-1α. Here we demonstrate that a synthetic peptide mapped on the C-terminus of SDF-1*β* presents inhibitory activity, whereas an analogue reproducing the C-terminal trait of SDF-1α does not show any activity. The opposite biological effect of the two peptides correlates with the type of interaction they each have with heparin and chondroitin sulfate.

Introduction

The chemokine stromal cell-derived factor-1 (SDF-1) is a member of the chemokine superfamily of pro-inflammatory mediators; it functions as a growth factor and a potent chemoattractant through the activation of CXCR4 as its natural ligand. CXCR4, a G-protein-coupled receptor, is also the co-receptor for X4 strains of human immunodeficiency virus type 1 (HIV-1). HIV-1 entry into target cells requires the interaction between its envelope glycoprotein gp120 and two membrane cellular receptors, the CD4 molecule and a chemokine receptor, usually CXCR4 or CCR5.[1]

SDF-1 is produced in three forms (SDF-1α, SDF-1*β*, and SDF-1*γ*) by alternative splicing of a single gene.[2] The difference between SDF-1α and SDF-1*β* lies in four additional C-terminal amino acids in the SDF-1*β* sequence. SDF-1*γ* contains a more extensive C-terminal sequence that has a different amino acid composition than the SDF-1*β* C-terminal one.

Crystallographic and NMR[3] data reveal that the SDF-1α monomer has a conventional chemokine topology, consisting of three major domains: the N-terminus, characterized by mostly extended conformation, three antiparallel *β*-strands, and a C-terminal α-helix that is packed against the *β*-sheet. The SDF-1*β* NMR characterization reveals no significant change in either secondary or tertiary structure with respect to SDF-1α.[4]

SDF-1α and SDF-1*β* have been shown to inhibit the infection of CD4+ cells by X4 HIV-1 strains.[5] The precise mechanism by which SDF-1 interferes with virus entry into cells is unknown. The hypotheses about the antiviral activity mechanism are (i) competition for the binding of gp120 to chemokine receptors due to CXCR4 occupancy by SDF-1,[2] (ii) down-regulation of CXCR4,[6,7] and (iii) receptor dimerization.[6]

The N-terminal region of SDF-1α (residues 1−18) forms an important receptor site in which Lys^1 and Pro^2 are residues directly involved in receptor activation. In addition, the RFFESH motif (residues 12−17) seems to be an important initial docking site of SDF-1 with CXCR4. The antiviral and signaling properties of SDF-1 (IC_{50} = 79 nM) are retained, in part, by a peptide corresponding to its amino terminus (sequence 1−13; $IC_{50} = 22 \pm 2$ μM); the single modification $Leu^5 \rightarrow$His gives an analogue that induces neither significant signaling nor desensitization of CXCR4 but retains antiviral activity (IC_{50} = 4 μM) superior to wild-type peptides.[8]

Some of the biological activities of chemokines are thought to be influenced by their association with cellular or matrix extracellular glycosamminoglycans (GAG), i.e., heparin, heparan sulfate, dermatan sulfate, and chondroitin sulfate.[2] In fact, it was shown that the optimal inhibition of X4 strains by SDF-1 requires interaction between SDF-1 and GAGs, probably because the chemokine−GAG binding increases the local concentration of SDF-1 in the surrounding environment of CXCR4.[2,9] A cluster of basic residues in the first *β*-strand, Lys^{24}, His^{25}, and Lys^{27}, represents the identified GAG-binding site on SDF-1α.[2]

Despite several studies on SDF activity, the role of the SDF-1 C-terminal region remains unclear. The attachment of the SDF-1α C-terminal sequence (residues 55−67) to the N-terminal one (residues 5−14) only enhances the induction of the calcium influx of the N-terminal peptide.[10] The binding of the C-terminal region of SDF-1α with heparin parallels the interaction between GAGs and the C-terminal helices of MCP-1 and IL-8 chemokines.

Using a similar approach, C. Tudan et al. demonstrated that the N-terminus is required for SDF-1 to

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10.1021/jm031067a CCC: $27.50
Published on Web 05/07/2004

Table 1. Sequences of Peptides Patterned on SDF-1β and SDF-1α

name	derivation	sequence																					
SDF-1βT	(51−72) SDF-1β	I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K	R	F	K	M
SDF-1αT	(51−68) SDF-1α	I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K				
SDF-1βT Nal^{70}		I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K	R	Nal	K	M

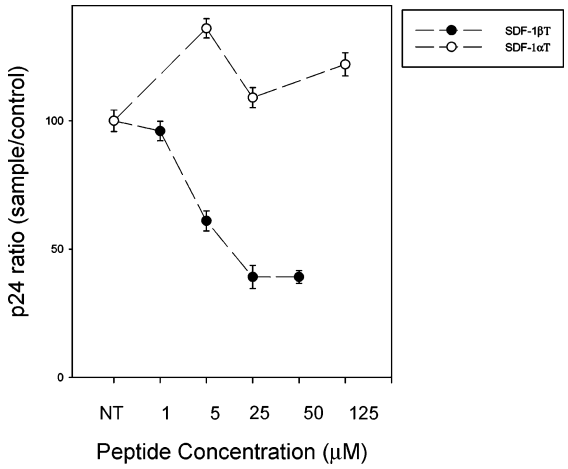


Figure 1. Effect of the peptides SDF-1αT (○) and SDF-1βT (●) on the X4 HIV-1 infection. U87MG-CXCR4 cells were incubated with serial 2-fold dilutions of each peptide for 30 min at 37 °C and then infected with HIV-1 $_{IIIB}$. Culture supernatants were removed after 24 h and replaced with fresh medium without peptide. The viral infection was evaluated by determining p24 antigen levels in culture supernatants 3 days post-infection. The data are plotted as the ratio of peptide-treated sample/peptide-untreated control p24 values and show the mean values of four separate experiments.

bind to CXCR4, but its affinity for its receptor is increased thanks to the C-terminus, which requires an α-helical conformation.[11]

Despite the recognized importance of the N-terminal trait in determining SDF anti-HIV-1 activity, SDF-1β, which has an extra C-terminal trait, has stronger antiviral activity than SDF-1α.[4] Consequently, we investigated and compared the antiviral activity of peptides reproducing the C-terminal region of SDF-1α and SDF-1β, respectively. The anti-HIV activity of the peptide mapped on the SDF-1β C-terminal site led us to design some peptide analogues in order to increase their inhibitory activity.

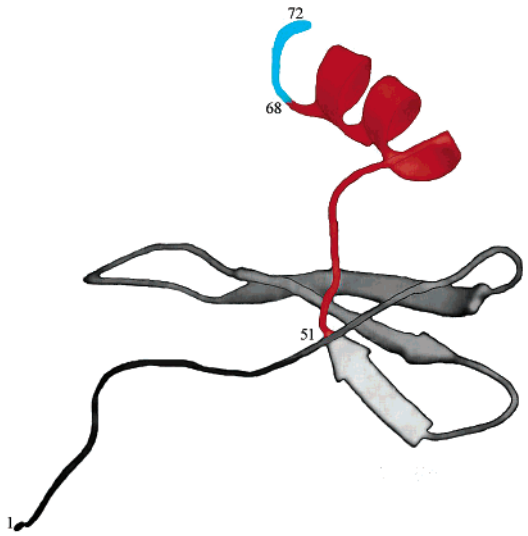
Results

SDF-1 is the natural ligand of CXCR4, the principal coreceptor for HIV-1 X4 isolates. SDF-1 is produced in three forms, namely SDF-1α, SDF-1β, and SDF-1γ. Both SDF-1β and SDF-1α are able to inhibit entry and replication of X4 HIV-1 strains.

Considering that the molecules SDF-1α and SDF-1β show an identical sequence, except for four additional C-terminal residues, and that this difference in length determines a 2-fold more potent inhibitory capacity for SDF-1β with respect to the shorter molecule SDF-1α,[4] we have here proposed the study of anti-HIV-1 activity and structural investigation of peptides reproducing the C-terminal sequence of SDF-1β (peptide SDF-1βT) and SDF-1α (peptide SDF-1αT) (Table 1), to clarify the role of the C-terminal trait of such chemokines. As shown in Figure 1, the peptide SDF-1βT inhibits the infection of X4 prototype HIV-1$_{IIIB}$ on the CXCR4+ cells in a dose-dependent manner. At a concentration of 25 μM, the peptide SDF-1βT reduces the p24 antigen level by 71% in comparison to untreated cells. Interestingly, in the same virus/cell system, the peptide SDF-1αT does not show any antiviral activity.

It was suggested that a possible motive for the difference between SDF-1α and SDF-1β HIV antiviral activities is the bulkiness of the C-terminal region;[4] consequently, to promote an increase in SDF-1βT antiviral activity, we designed an analogue named SDF-1βT Nal^{70} (Table 1). The rationale behind substitution Phe^{70}→Nal was to increase the bulkiness of the C-terminal region of the peptide. However, the results of the biological assays (data not shown) demonstrated that the SDF-1βT Nal^{70} analogue cannot increase or retain the inhibitory activity of SDF-1βT.

The SDF-1αT and SDF-1βT solution conformations were investigated by carrying out CD analyses in different solvent systems (phosphate buffer solution at pH 7, water, tetrafluoroethylene (TFE), sodium docecyl sulfate (SDS) solution). In the phosphate buffer, the peptide SDF-1αT shows an α-helix-like spectrum, characterized by a maximum at 188 nm and two minima at 204 and 220 nm of modest intensities (Figure 2A). The helical content estimated using the ellipticity value at 220 nm, according to Greenfield and Fasman,[13] is 15%. It must be pointed out that the peptides in question comprise only the helical region of the SDF-1 structure,



and so the estimation of helix content from the amplitude of the 222 nm negative band alone is probably sufficient. In the same solvent, the CD spectrum of SDF-1βT (Figure 2A) has a similar pattern characterized by the red shift of positive and negative bands toward canonical α-helical values (190, 208, and 222 nm). The spectrum presents more intense bands; consequently, the estimated α-helix percentage rises to 29%.[13] In the phosphate buffer, the analogue SDF-1βT Nal^{70} (Figure 2A) shows a more intense α-helical CD spectrum than SDF-1βT (estimated α-helix content = 57%). In the

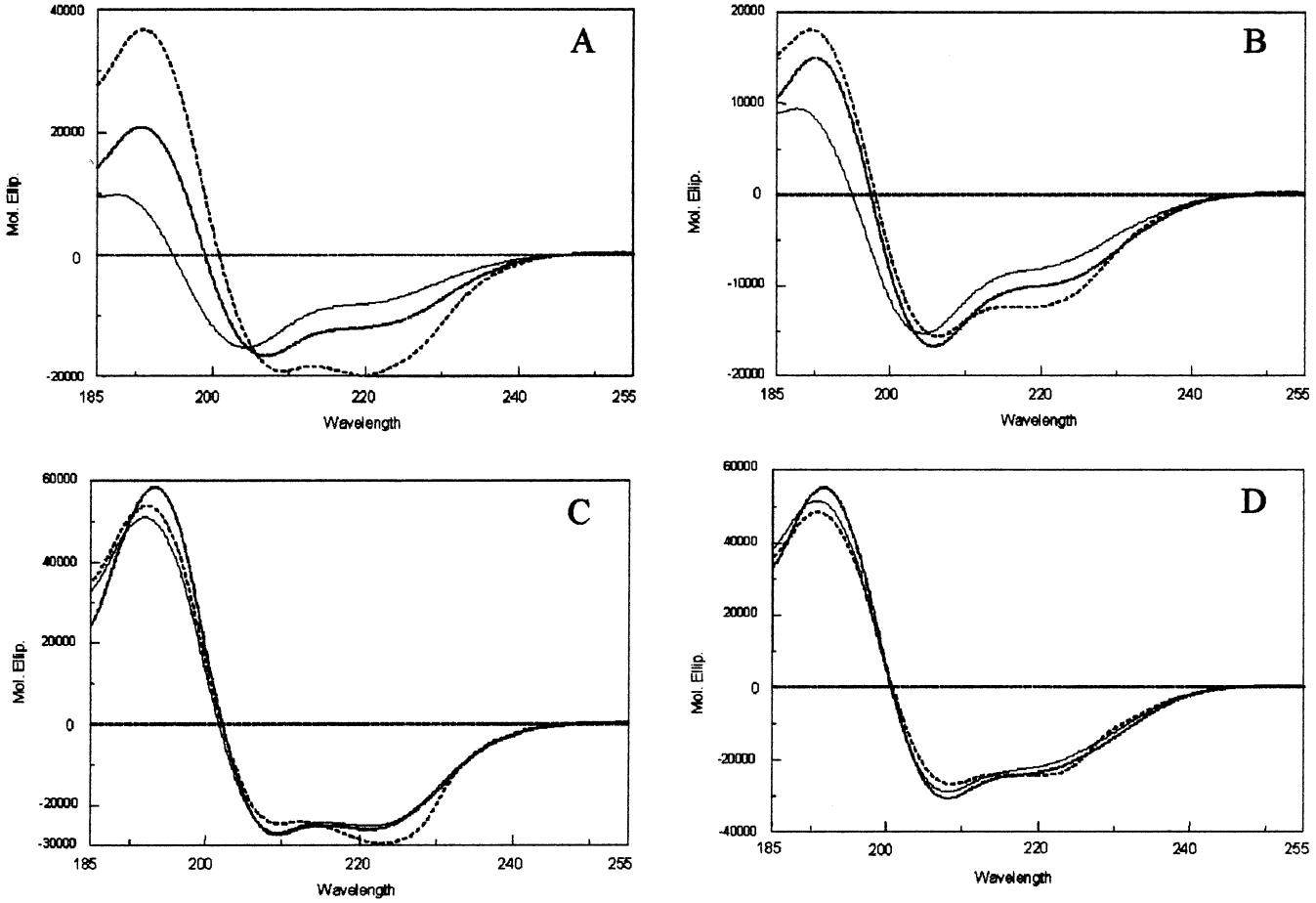


Figure 2. CD spectra of peptides SDF-1αT (—), SDF-1βT (···), and SDF-1βT Nal[70] (- - -) in 10 mM phosphate buffer, pH 7 (A); H_2O (B); 14 mM SDS, 10 mM phosphate buffer, pH 7 (C); and TFE (D).

aqueous solution, characterized by a more acidic pH, the CD profiles do not change substantially (Figure 2B). In the SDS solution, the α-helical pattern is confirmed for all the CD spectra (Figure 2C). The SDF-1αT and SDF-1βT spectra at $\lambda > 200$ nm show clash patterns (estimated α-helix content = 76−80%), whereas the spectrum of analogue SDF-1βT Nal[70] is characterized by a negative band at 223 nm that is more intense than the negative band at 208 nm (estimated α-helix content = 88%). The α-helix increases in SDS solution, with respect to the aqueous solution, might be ascribed to the stabilization of amphipatic helices in micelles. In a helical promoting solvent, such as TFE, the different peptides reach α-helix percentages similar or lower to those obtained in the SDS solution (Figure 2D) (SDF-1αT, 65%; SDF-1βT, 70%; SDF-1βT Nal,[70] 74%).

In the literature, there is increasing evidence that the biological activities of chemokines might be influenced by their association with cellular or extracellular matrix GAGs.[2] GAGs are highly sulfate oligosaccharides; the most common ones are heparin, heparan sulfate, chondroitin sulfate, and hyaluronic acid. An increasing body of evidence suggests that GAGs immobilize and enhance local concentrations of chemokines, promoting their oligomerization and facilitating their presentation to the receptors.[9] It has been generally assumed that the major interactions between chemokines and GAGs are electrostatic and involve the negatively charged sulfates and carboxylates on the GAGs and positively charged residues on the protein. In fact, the typical heparin-binding motif is BBXB, where B is a basic amino acid and X is a non-basic residue. Analyzing the sequences of SDF-1αT and SDF-1βT, we noted the presence of a BBXB motif (KRFK) in the C-terminal sequence of SDF-1βT, involving the residues that differentiate SDF-1β from SDF-1α. The hypothesis of specific interaction between the C-terminal region of SDF-1β and GAGs, absent in the SDF-1α C-terminal region, is substantiated by the analysis of the CD spectra in the presence of different concentrations of heparin and chondroitin sulfate. The results of the CD investigations are reported in Figure 3.

Titration with Heparin. In the aqueous solution, the CD spectra of all the peptides show an α-helix pattern; consequently, we estimated the intensity variation of a diagnostic band (at 220 nm) for growing additions of GAGs (Figure 3). Upon addition of 10 μM heparin, the band at 220 nm of the SDF-1βT CD spectrum decreases, ranging from 23% to 12% in α-helical content (Figure 3B). Subsequent additions of heparin cause a recovery of the ellipticity at 220 nm, with stable values over 400 μM. At 400 μM heparin, the α-helical content estimated for the CD spectrum of SDF-1βT is doubled in comparison with the initial value (Figure 3B).

The same experiment was carried out using SDF-1αT: the results reported in Figure 3A demonstrate that, in this case, there is no mutual relation between the 220 nm band intensity (correlated to peptide α-helical structure content) and the concentration of GAGs. On the other hand, the SDF-1βT Nal[70] analogue spec-

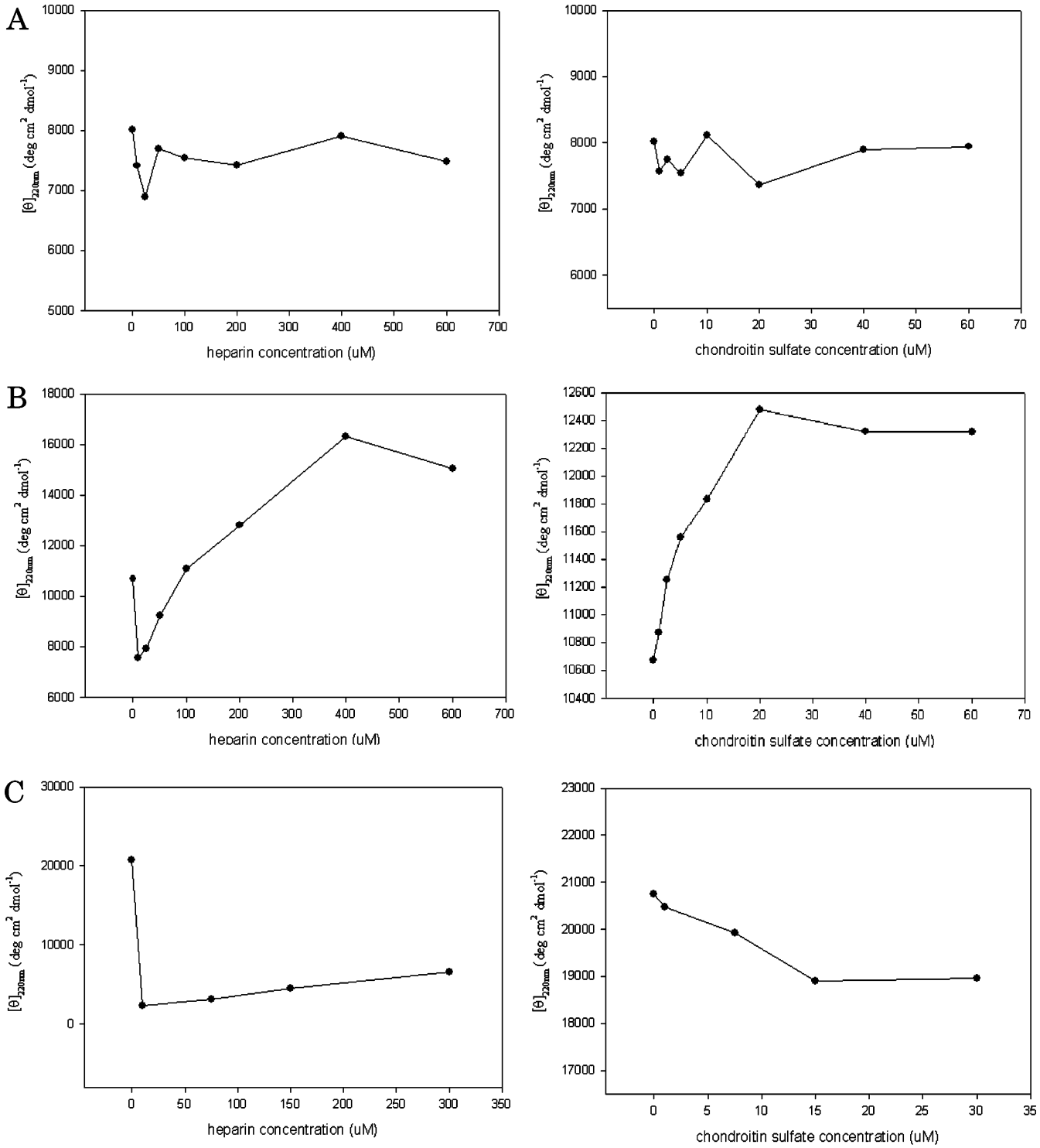


Figure 3. Change in the mean residue ellipticities at 220 nm for SDF-1αT (A), SDF-1βT (B), and SDF-1βT Nal[70] (C) incubated with increasing concentrations of heparin and chondroitin sulfate.

trum shows zero setting of the α-helical structure when 10 μM heparin is added (Figure 3C). Further heparin additions do not cause a refolding of the molecule in the α-helical form or the other well-characterized structures.

Considering that binding studies carried out using the entire SDF-1α with selectively desulfated heparin, heparin oligosaccharides, and heparitinase-resistant heparan sulfate have shown that a minimum of 12−14 monosaccharide units are required for efficient binding,[14] we had to consider that the reference concentration of heparin in our studies had to be over 400 μM. Consequently, at a 400 μM heparin concentration, we can observe that SDF-1βT shows double α-helical content in comparison with the untreated sample, SDF-1αT does not show any important variation consequent to the addition of GAGs, and the SDF-1βT Nal[70] spectrum loses its initial α-helical content.

Titration with Chondroitin Sulfate. The results of the titration with chondroitin sulfate (Figure 3) of the different peptides can be summarized as follows:

The addition of increasing amounts of chondroitin sulfate to the SDF-1βT solution creates a steady in-

crease in α-helical content, as demonstrated by the rise in the 220 nm band intensity that reaches the highest value at a ≥20 μM concentration of GAGs (Figure 3B).

The titration of SDF-1αT with chondroitin sulfate does not show any significant variations with respect to the spectrum without GAGs (Figure 3A).

The addition of chondroitin sulfate in the SDF-1βT Nal[70] solution causes a decrease in the value of the CD curve at 220 nm (Figure 3C); this can be correlated to the loss of α-helix content.

Discussion

The chemokine receptor CXCR4 is a CD4-associated coreceptor for X4 strains of HIV-1 and represents a target for antiviral therapy. Infection by X4-HIV-1 can be blocked by SDF-1, the natural ligand of CXCR4. Several studies have shown that the N-terminal region of SDF-1 is involved to a greater extent in the chemokine's antiviral activity.

The observation that the two forms of SDF-1, differing only in the C-terminus length,[2] show dissimilar antiviral activity led us to investigate and compare the role of the C-terminal region of SDF-1α and SDF-1β. Surprisingly, the C-terminal fragment of SDF-1β (residues 51−72) showed X4 HIV-1 inhibitory activity comparable to those reported for N-terminal fragments, whereas the peptide SDF-1αT (residues 51−68 of SDF-1α) showed no activity. This last finding agrees with similar results reported in the literature.[8] Consequently, we demonstrated the following:

(i) Despite the significant deletion, the peptide SDF-1βT is able to retain part of the protective activity of the native molecule.

(ii) Even though some researchers indicate the most important sites for binding and activity in the chemokine N-terminal region, our results seem to show that SDF-1β contains an additional region, with respect to SDF-1α, which may have important roles in anti-HIV activity.

(iii) The difference in activity between the two peptides correlates with the different inhibitory activity of the native chemokines SDF-1α and SDF-1β.

It seems unlikely that the biological activity of SDF-1βT might be ascribed to its interaction with CXCR4 and, consequently, to a direct competition with gp120 for CXCR4 occupancy. In fact, it is well-known that the residues that play a critical role in CXCR4 recognition and signal transduction lie in the N-terminal region of the molecule. Interestingly, all the evidence makes it clear that the biological properties of chemokines are influenced by their association with GAGs. In particular, surface plasmon resonance kinetic analysis shows that the CXC chemokine SDF-1α associates with relatively high affinity to heparin. Furthermore, it has been demonstrated that SDF-1α is able to bind CXCR4 negative epithelial or endothelial cells interacting selectively with GAGs.[15] The binding of chemokines to GAGs is mediated through ionic forces generated by the interactions of negatively charged side chains on GAGs with clusters of basic residues in chemokines. The residues responsible for the binding of the CC chemokines (MCP-1 and MIP-1α) to heparin are located in distinct domains outside the carboxy-terminal α-helix.[16,17] In contrast, mutagenic and biochemical studies of chemokines IL-8 and PF-4 have led to the conclusion that CXC chemokines may bind to heparin through a cluster of positively charged residues (mainly Lys) located in the C-terminal amphipathic α-helix.[18,19] Similar conclusions have been reached from an analysis of the carboxy-terminal α-helix of the chemokine GROα or NAP-2.[20] However, recent findings indicate that the interaction of CXC chemokines with heparin may be mediated by domains other than the C-terminal α-helix. In PF-4, a loop containing five positively charged residues contributes more than previously identified C-terminal end Lys residues to the interaction of the chemokine with heparin.[21] This situation is similar to that of SDF-1α. In addition, studies of the electrostatic potential reveal that the C-terminal α-helix of SDF-1α possesses a predominant negative surface charge[4] that makes its relevance as a potential GAGs binding site very unlikely. On the other hand, the basic residues Lys[24], His[25], Lys[27], and Arg[41] clustered along the two first β-strands exhibit a high, positive potential and are a suitable region for interaction with GAGs.

Unlike the C-terminal region of SDF-1α, the last residues of the C-terminal α-helix of SDF-1β possess positive charges.

In particular, we observed a BBXB motif in the 68−71 sequence of SDF-1β that is considered typical of heparin binding sequences. To ascertain the capacity of SDF-1βT to bind GAGs, we carried out some titration experiments using heparin and chondroitin sulfate. The results showed that, in 400 μM heparin, the peptide SDF-1βT doubles its α-helix content while the conformation of SDF-1αT does not change. In a study about modeling the protein−heparin interaction, it was suggested that the reason for the α-helix enhancement is the stabilization of the helix by the electronic interaction between side-chain positive peptide residues and negative groups of GAGs.[22] This interaction is influenced by the type and number of charges of each GAG; consequently, the results of peptide interaction with heparin or chondroitin sulfate are variable. The analogue SDF-1βT Nal[70] does not have any HIV-1 inhibitory activity. This might be due to (i) the loss of a specific interaction with Phe[70], (ii) a change in the peptide's biologically active conformation, or (iii) steric hindrance. The second hypothesis is unlikely, since the SDF-1βT Nal[70] CD spectrum presents more intense α-helix bands than the SDF-1βT CD spectrum. Interestingly, this more marked helical structure is completely suppressed in the presence of heparin, whereas SDF-1βT increases its α-helical structure when 400 μM heparin is added. Consequently, the mutation of a single residue causes a remarkable decrease in the α-helix conformation in the presence of GAGs. In addition, an enhanced concentration of chondroitin sulfate drives SDF-1βT to a more stable α-helix structure, as well. The same GAG does not induce any relevant variation in the spectrum of SDF-1αT, nor does it cause a decrease in the helical content of the SDF-1βT Nal[70] spectrum. In conclusion, it is likely that SDF-1βT might interact with GAGs through the BBXB motif of its C-terminal region, differently from the behaviors of SDF-1αT and SDF-1βT Nal[70] analogues. The C-terminal α-helix of SDF-1β probably contributes to the binding to GAG molecules, which increases the local concentration of chemokines, promoting their oligomer-

ization and facilitating their presentation to the receptor. On the other hand, it is unlikely that the anti-HIV activity of the peptide SDF-1βT is due to the binding to CXCR4; rather, it is probably related to its capacity to interact with GAGs. Even if this is the case, the anti-HIV-1 effect of the C-terminal peptides is not an insignificant observation, since HIV-1 itself is believed to interact with cell-surface GAGs, and GAG-binding compounds, in principle, may compete with the virus. The C-terminal peptides might be useful in making other anti-HIV-1 agents stronger.

The antiviral effect of SDF-1βT is specific for X4 strains. No antiviral activity was observed when this peptide was used in infection experiments performed with R5 prototype HIV-1_{Bal} on U87MG-CCR5 cells (data not shown). This effect is probably due to the different strain-dependent use of GAGs. Accordingly, it was recently demonstrated that cell-surface GAG-mediated effects on virion attachment and infection vary in an envelope strain-dependent manner and are enhanced in isolates, such as X4, that contain a high, positively charged V3 loop sequence.[23]

Experimental Procedures

Materials. Fmoc-protected amino acids and resins were obtained from Novabiochem (Langelfingen, Switzerland); 0.45 M HBTU/HOBt solution in DMF from Applied Biosystems (Perkin-Elmer, Norwalk, CT); MeCN, NMP, DCM, and TFA from Janssen (Geel, Belgium); and piperidine, DIEA, 1,2-ethandithiol, and thioanisole from Aldrich (Deisenhofen, Germany). Dulbecco's modified Eagle's medium was obtained from Life Technologies Gibco BRL (Gaithersburg, MD), gentamicyn from Sigma (Milan, Italy), 2% glutamine and 10% FCS from Gibco (Tulsa, OK), and ELISA test from NEN Life Science Products (Boston, MA). The heparin and chodroitin sulfate C were provided by Sigma (Milan, Italy).

Peptide Synthesis. To synthesize SDF-1 C-terminal peptides and their analogues (sequences reported in Table 1), the Fmoc strategy was carried out using an Applied Biosystems model 431A automatic synthesizer. The resins used were HMP (SDF-1βT), Fmoc-Met-Wang resin (SDF-1βT Nal^{70}), and Fmoc-Lys(Boc)-Wang resin (SDF-1αT). HBTU was used as a coupling reagent. The side-chain protections used were as follow: for Asp and Glu, OtBu; for Lys and Trp, Boc; for Gln and Asn, Trt; for Tyr, tBu; and for Arg, Pmc. Double coupling was done for all insertions in the case of SDF-1βT, SDF-1αT, and SDF-1βT Nal^{70} syntheses. Specifically for the SDF-1βT synthesis, after each single coupling step, a resin sample was subjected to a quantitative ninhydrin test: the extent of the coupling was particularly low for the Lys^{68} (96.1%), Lys^{64} (96.2%), Leu^{55} (96.3%), and Lys^{54} (96.0%) insertions. The cleavage from the solid support and the deprotection of the side chains were achieved using the following mixture: 0.75 g of phenol, ethanedithiole:thioanisole:H_2O:trifluoroacetic acid = 0.25:0.5:0.5:10 over 1.5 h. The purification of the crude products was obtained using reversed-phase HPLC. The final analytical chromatogram of SDF-1βT on a Vydac C_{18} column (5 μm, 300 Å, 4.6 × 250 mm) gave a 98.9% purity grade and t_R = 14.3 min under the following conditions: eluent A, 0.05% TFA in water; eluent B, 0.05% TFA in MeCN; flow rate, 1 mL/min; gradient 30−40% B over 20 min; detector, 214 nm. The homogeneity grade of the purified peptides was 97.2% for SDF-1αT and 99.5% for SDF-1βT Nal.[70] The data were obtained by integrating the chromatographic pattern of each purified peptide obtained using the above-reported conditions and the following gradients: 28−38% B over 20 min for SDF-1αT (t_R = 13.4 min) and 30−40% B over 20 min for SDF-1βT Nal^{70} (t_R = 14.8 min). The identity of the purified products was determined using MALDI spectrometry (SDF-1βT theoretical value 2792 Da, experimental value 2792 Da; SDF-1αT theoretical value 2229 Da, experimental value 2229 Da; SDF-1βT Nal^{70} theoretical value 2841 Da, experimental value 2845 Da).

Biological Assays. HIV-1 infection experiments were performed using human glioblastoma cell lines stably expressing CD4 and CXCR4 (U87MG-CXCR4).[12] The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 50 μg/mL gentamicyn, 2% glutamine, and 10% FCS. For infection assays, cells were seeded in 48-well plates (Falcon) at a concentration of 5×10^3 cells/well for 20 h before infection. The cells were washed twice with fresh medium, pretreated with increasing doses of different peptides for 30 min at 37 °C, and then infected with X4 prototype HIV-1_{IIIB} (5000 pg of p24 protein equivalent/well). To monitor viral infection, the supernatants were harvested 3 days after infection, and the determination of the HIV-1 p24 antigen was performed using the ELISA test.

Circular Dichroism. CD spectra were collected at room temperature using a Jasco model J710 automatic recording circular dichrograph. Cylindrical, fused quartz cell with a 0.1 cm path length was used. The spectra are reported in units of mean residue ellipticity (peptide molecular weight/number of amide bonds), $[\Theta]_R$ (deg cm^2 dmol^{-1}). For wavelegth scans, the spectra were recorded from 250 to 180 nm for the peptide samples, and from 260 to 200 nm for the peptide samples conditioned with glycosaminoglycans, with a step size of 0.2 nm and bandwidth of 2 nm, corrected for the buffer, water, or TFE baseline and SDS or GAG contributions measured in the same cells.

The peptide solutions were prepared by dissolving a weighed quantity of peptide in the minimum amount of water to which 10 mM phosphate buffer, pH 7, water, a micellar solution (14 mM SDS in 10 mM phosphate buffer, pH 7), or TFE was added, giving a final content of 99% (v/v).

The peptide and GAG solutions were prepared by dissolving a weighed quantity of peptide in a solution buffer (0.15 M NaCl, 0.01 M phosphate buffer, pH 7.4) to which increasing amounts of heparin (10−400 μM) or chondroitin sufate (1−40 μM) were added. For chodroitin sulfate experiments, a concentration range 10-fold less than that of the other GAGs was used, since this GAG has a 10-fold higher molecular weight. Peptide concentrations, determined by spectrophotometric analysis, ranged from 3.12×10^{-5} to 3.88×10^{-5} M.

Acknowledgment. This work was supported by grants from the Italian Ministry of Health, Progetto AIDS, Istituto Superiore di Sanità.

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JM031067A