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Synthetic Peptides for Study of Human Immunodeficiency Virus Infection

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Abstract

The formation of a complex among gp120, CD4, and CCR5/CXCR4 represents a key step in human immunodeficiency virus (HIV) infection. The use of synthetic peptides reproducing sequences of these surface proteins has increased knowledge about the interactions that determine the penetration of HIV viruses into target cells. The final aim of such investigations is the design of molecules able to inhibit the initial step of infection and the development of high-sensitivity in vitro assays for detection of HIV. In particular, the studies presented herein concern the role of the gp120 V3 loop in the CD4 binding, the importance of the N-terminal sequence of HIV-coreceptor CCR5, the sequences patterned on CXCR4 natural ligand (stromal-derived factor 1 [SDF-1]) as inhibitory peptides, and the importance of substrate secondary structure in determining the enzymatic processing of gp120 precursor (gp160).

Index Entries: Synthetic peptides; human immunodeficiency virus; gp120-CD4 binding; SDF-1; CCR5; CXCR4; drug design.

Introduction

An important key step in human immunodeficiency virus (HIV) infection is represented by the binding between the viral envelope glycoprotein gp120 and the surface antigen CD4 of T-cells *(1)*. The binding involves two principal sites but is not sufficient by itself to allow the virus entry into target cells. In fact, in human cells expressing CD4, HIV-1 binds, but virus-host membrane fusion does not occur *(2,3)*. In addition, the tropism of HIV (T-tropic and M-tropic strains) suggests that an accessory factor may be required, in addition to CD4, to elicit the correct alterations in Env that lead to membrane fusion. For these reasons, the existence of cofactors that function in conjunction with CD4 was postulated, more than 10 yr ago.

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The first chemokine receptor, individuated as a coreceptor for HIV, was CXCR4 *(4)*; it has been shown to specifically mediate T-tropic viral fusion but not as a coreceptor for the M-tropic viruses. Sequence analysis reveals that CXCR4 is a member of the superfamily of G-protein-coupled receptors containing seven transmembrane segments (STD) and, more precisely, of the family of the CXC receptors. The discovery of CXCR4, together with the observation that CC-type chemokines such as RANTES, macrophage inflammatory protein (MIP)-1α, and MIP-1β inhibit infection by M-tropic HIV-1 viruses, strongly suggests that other chemokine receptors might act as coreceptor for HIV. Consequently, six receptors for the CC family of chemokines have been identified; among these, the CCR5 receptor *(5–7)* appears to be the major coreceptor for the M-tropic strains of HIV-1. The natural ligand of CXCR4 is the chemokine named SDF-1, which is able to inhibit the entry and replication of syncytium-inducing forms of HIV.

In addition, recent studies indicated that the V3 domain of gp120 determines the coreceptor to be used. All this suggests that chemokine production, on the one hand, and coreceptor expression, on the other, could represent two very important biologic variables that determine HIV-1 entry into a specific part of the organism, the velocity of infection, and its course. The discovery of the HIV coreceptors could result in the development of new therapeutic agents capable of blocking virus access to the coreceptor. These small molecules endowed with inhibitory properties could be used in place of chemokines. In this article, we summarize our studies concerning the biologic activity of synthetic peptides reproducing sequences of gp120 V3 loop, CCR5, CXCR4, and SDF-1. The final aim of our investigations was the design of molecules able to inhibit the initial step of infection and the development of high-sensitivity in vitro assays for the detection of HIV. Another interesting key event in the biosynthesis of HIV is the maturation of the gp160 precursor generating gp120 and gp41 *(8)*, two proteins that are fundamental for the infective process. Such a process is essential for infection because gp160 is not functional. We are studying the conformational structure of a peptide, reproducing the 505–523 sequence of gp160, that is correctly processed in vitro by furin, i.e., the endoprotease involved in gp160 processing. Such investigations could help in the development of specific inhibitors of the enzyme that could block the gp160 maturation process.

Discussion

Peptides Patterned on V3 Loop of gp120

A peptide of 23 amino acids, named DB3 (Table 1, bold sequence), derived from the V3 loop of the surface glycoprotein of HIV-1 MN strain was able to bind soluble CD4 and enhance HIV-1 infection in a dose-dependent and nonvirus-restricted manner *(9,10)*. The mechanism and structural features required for these biologic activities were studied by using shortened DB3 derivatives and DB3 analogs carrying single amino acid substi-

Table 1
Sequences of Peptides Patterned on gp120 V3 Loop
of Different HIV-1 Strains and Their Analogs

Derivation	Sequence[a]
IIIB	N N T R K S I R I Q R G P G R A F V T I G I G
RF	N N T R K S I R I T K G P G R V I Y A T Q I
BAL	N N T R K S I H I G P G R A F Y T T G E I I G
BALSC1	I T Y G I T I S A F N R T Q I G R N H G P G
BALSC2	T F I A E I H R Y P T N G R I G I S G G T N K
BAL-R	N N T R K S I H I G P G R A F Y T T G R I I G
MN	**Y N K R K R I H I G P G R A F Y T T K N I I G**
DB3	Y N N R K R I H I G P G R A F Y T T K N I I G
DB3	Y N K R K N I H I G P G R A F Y T T K N I I G
DB3	Y N K R K R I H I G P G R A F Y T T N N I I G
DB3	Y N K R K R I H I G P G R A I Y T T K N I I G
DB3	Y N K R K R I H I G P G R A F I T T K N I I G
DB3	Y N K R K R I H I G P G R A F Y T T K N I I G
DB3	Y N K R K R I H I G P G R A F Y T T K N I I G
DB3	Y N K R K R I H I G P G R A F Y T T
DB3	I H I G P G R A F Y T T
DB3	G P G R A F Y T T

[a]Underlined residues represent D-amino acids.

tutions (Table 1). We found that peptides in which the aromatic amino acids in position 15 or 16 had been replaced by an uncharged hydrophobic residue, analogs in which positively charged amino acids were replaced by corresponding D-enantiomers, and shortened DB3 derivatives lost both enhancing activity and the ability to bind to soluble CD4 *(11)*. Other peptide variants in which a positively charged amino acid was replaced by asparagine at positions 3, 6, and 19, respectively (Table 1), retained both enhancing and binding activities, although with different efficiencies *(11)*. Peptide DB3 enhanced CD4 expression on peptide-treated cells as well as gp120 binding to both $CD4^+$ cells and soluble CD4. These findings strongly suggest that the peptide/CD4 interaction induces an increase in both CD4 expression and CD4/gp120 binding affinity, which, in turn, mediates the enhancement of viral infection.

These results have two important practical applications. First, the possibility of using the antigenic sequences of the viral V3 loop to design a vaccine for HIV infection must be reviewed considering the biologic activity of peptide DB3. Second, several trials clearly confirmed that the peptide DB3 is suitable for the direct diagnosis of HIV-1 *(12–14)*. In fact the peptide produces a well-detectable increase in syncytia (giant cells characteristic of HIV infection) formation in infected cell culture. Consequently, the molecule could be used to determine the presence of HIV in patients who are not yet producing anti-HIV antibodies. The use of a new diagnostic test, including among its reagents the peptide DB3, could resolve the prob-

lem of false-negative response owing to the window period between the infection and the production of antibodies that are detected by the diagnostic tests currently in use.

Importance of the N-Terminal Sequence of HIV Coreceptor

We demonstrated that a peptide reproducing the sequence 1–26 of CCR5 is able to increase 50 times, in a dose-dependent manner, the infection of HIV-IIIB in an experiment using U87MG CXCR4$^+$ cells (unpublished results). Radioimmunoassay tests showed that the peptide (1–26)CCR5 is able to bind to sCD4 (unpublished results); this finding is in line with recent published results about the association between CCR5 and CD4 in the absence of gp120 and the importance of CCR5-CD4 interaction in the formation of gp120-CD4-CCR5 complex *(15)*. Since, more recently, different research groups have demonstrated that specific amino acids, including acidic residues and tyrosines and in particular Tyr^{15}, located within the CCR5 amino-terminal domain, are essential for CCR5-mediated fusion and HIV-1 entry *(16)*; and that residues 3, 10, and 14 might be sulfated and several sulfated compounds can inhibit HIV-1 entry *(17)*, we have synthesized two 26-mer peptides, the first carrying a Tyr^{15} → Ala modification and the second containing sulfotyrosines in positions 3, 10, and 14. The last analog might serve to explain whether the switch between inhibition and enhancement is owing to the posttranslational modification of tyrosines. The aim of these studies is the design of a drug able to inhibit the interaction of gp120 to the coreceptor of target cells.

Anti-HIV Activity of Synthetic Peptides Derived from SDF-1

The natural ligand of the coreceptor CXCR4 is the chemokine SDF-1 that is able to inhibit the entry and replication of syncytia inducing forms of HIV *(7)*. This molecule is physiologically produced as SDF-1α (68mer) and SDF-1β (72mer); the four additional residues are located in the C-terminus. Despite the presence of the binding site in the N-terminus of the molecule, SDF-1β is twofold better than SDF-1α in inhibiting the virus. Accordingly, we are studying the inhibitory activity of peptides reproducing the C-terminal region of SDF-1β. Interestingly, the peptide (46–67)SDF-1β has shown anti-HIV activity in experiments using U87MG-CXCR4 cells and HIV-1-IIIB, suggesting that other regions of the molecule, in addition to the N-terminal region, may participate in the binding *(18)*. Investigations on the role of the four C-terminal residues in determining the anti-HIV activity of SDF-1β and its fragments using point mutated analogs (Table 2) are in progress.

Gp160 Processing

The maturation process by which the inactive viral glycoprotein gp160 is transformed into two glycoproteins, named gp120 and gp41, is a crucial step for the binding and penetration of the virus into the target cells.

Table 2
Sequences of Peptides Patterned on SDF-1β and SDF-1α

Name	Sequence[a]																								
(46-67) SDF-1β	I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K	R	F	K	M			
(46-63) SDF-1α	I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K							
(46-66) SDF-1β Phe^{65}→Nal	I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K	R	Nal	K				
(46-65) SDF-1β Phe^{65}→Nal	I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K	R	Nal					
(46-67) SDF-1β Phe^{65}→Nal	I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K	R	Nal	K	M			
(46-67) SDF-1β Met^{67}→Nle	I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K	R	F	K	Nle			
(46-67) SDF-1β Met^{67}→Nle Phe^{65}→D-Phe	I	D	P	K	L	K	W	I	Q	E	Y	L	E	K	A	L	N	K	R	F	K	Nle			

[a]Underlined residues represent D-amino acids.

The enzymatic digestion of synthetic peptides modeled around the cleavage site (sequence 505–523 of gp160) has provided interesting information about the role of single residues and of secondary structure requirements. For example, nuclear magnetic resonance data and restrained molecular dynamics (RMD) simulations indicate the presence of a helical segment at the N-terminus, whereas the C-terminus, including the processing site, is unstructured and can be described as a loop, presenting bending exposing the processing site (unpublished results); the structure assumed by the gp160 cleavage site might be the classic helix-loop-helix motif. In addition, the introduction of two Pro residues at positions 516 and 520 improves substrate recognition and processing by the specific enzyme (unpublished results). The effect seems to be the balance between the positive Pro^{516} substitution and the negative Pro^{520} one. In particular, single-point modification at position 520 gives a poor substrate, whereas modification at position 516 results in the best substrates among all the proposed peptides.

Conclusion

Commonly used antiretroviral therapy targets both reverse transcriptase and protease enzymes by using specific nucleoside-like inhibitors such as AZT, ddc, ddl, and nonnucleoside inhibitors such as nevirapine and loviride. Both types of molecules suppress HIV replication but they do not prevent the creation of new viruses. New research is investigating ways of stopping HIV from binding and entering human CD4 cells. In this context, the use of synthetic peptides seems particularly promising, and it is hoped that "entry blocks or inhibitors" of peptide nature will soon be added to our choice of anti-HIV drugs. According to this "second wave" approach, we have been investigating several peptide molecules involved in the binding of HIV-1 and its target cells. These studies have led to the identification of peptides derived from the V3 loop of the gp120 that, enhancing infection, can be used for the direct detection of the virus, thereby finding interesting practical applications in the diagnostic field. In addition, peptide (46–67)SDF-1β has shown anti-HIV activity, suggesting that the C-terminus of the chemokine may participate in the binding to CXCR4. Finally, the use of model synthetic peptides reproducing the cleavage sites of the gp160 precursor of the functionally relevant gp120 and gp41 glycoproteins has allowed us to pinpoint the role of interesting structural motifs presiding over the maturation of the precursor by the specific enzyme.

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