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HIV gp120 Interactions with Coreceptors: Insights from Studies with CCR5-based Peptides

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Abstract
Human immunodeficiency virus enters cells by a direct fusion mechanism triggered by sequential binding of the gp120 subunit of the envelope glycoprotein, first to CD4, then to the coreceptor CCR5 or CXCR4. The coreceptors are chemokine receptors, members of the superfamily of G protein-coupled receptors that are characterized by 7 transmembrane domains. gp120 is presumed to interact with the extracellular portion, which consists of the N-terminal segment and three extracellular loops. Synthetic peptides based on these regions have proven to be valuable probes for elucidating the molecular details of the complex gp120-coreceptor interactions.

Key words: HIV, AIDS, coreceptor, CCR5, CXCR4, chemokine receptor, CD4, GPCR, peptides, fusion, entry

Abbreviations: HIV, human immunodeficiency virus; Env, envelope glycoprotein; GPCR, G protein-coupled receptor; ECL, extracellular loop

Introduction: Receptors Involved in HIV Entry

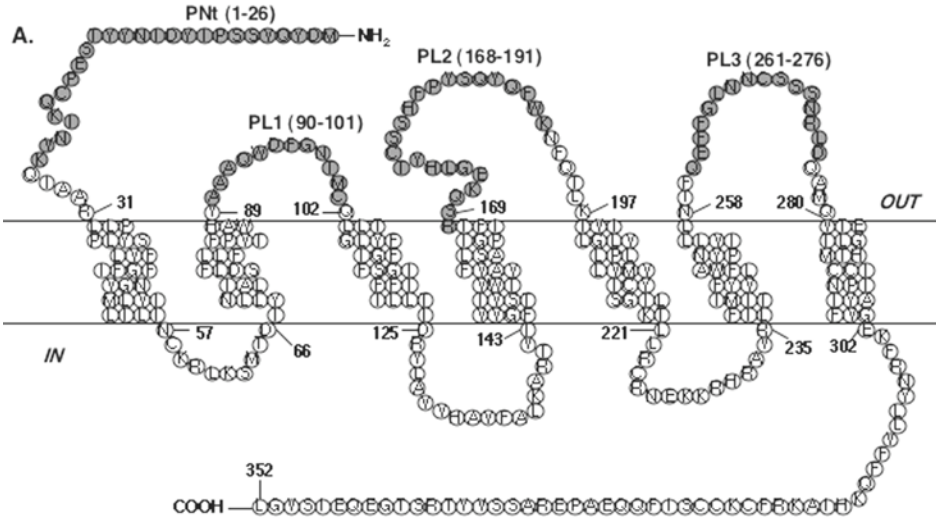
The surface of human immunodeficiency virus (HIV) is studded with spikes composed of the envelope glycoprotein (Env), whose function is to promote HIV entry by a process of direct fusion between the virion membrane and the plasma membrane of the target cell [1, 2]. Env is composed of two noncovalently associated subunits derived by proteolytic processing of the gp160 biosynthetic precursor: the gp120 external subunit, which is responsible for binding to specific target cell receptors, and the gp41 transmembrane subunit, which catalyzes the fusion reaction and anchors Env to the virion surface. The functional spike on the surface of virions or infected cells is organized as a trimer of three gp120-gp41 heterodimers. The HIV fusion/ entry reaction is initiated by sequential receptor binding of gp120, first to CD4 (the "primary receptor") and then to a specific chemokine receptor (the "coreceptor"), generally CCR5 or CXCR4 [3]. These receptor interactions then trigger gp41 to promote membrane fusion; this reaction is thought to involve extension of the gp41 subunit to allow insertion of its N-terminal "fusion peptide" into the target cell membrane, followed by refolding the pre-fusion intermediated into an energetically favorable 6-helix bundle that brings the two membranes together so that fusion can occur.

Chemokine receptors are structurally related molecules belonging to the superfamily of G-protein coupled receptors (GPCRs) [4-6]. GPCRs have been implicated in an extraordinary range of physiological functions and pathological activities, and have proven to be exceptionally important targets for drug development. Indeed diverse strategies focusing on the HIV coreceptors are under development for treatment and prevention of infection [7-9]. Like all GPCRs, the chemokine receptors are integral membrane proteins characterized by seven transmembrane helices, with an extracellular amino terminus, alternating intracellular and extracellular loops (three of each), and an intracellular carboxy terminal tail.

In their function as HIV coreceptors, CCR5 and CXCR4 physically associate with CD4-activated gp120, as shown by direct binding and co-precipitation studies [10-13] and by functional assays [14]. Unlike the requirements for chemokine receptor activity, the membrane fusion reaction requires neither G protein signalling nor coreceptor internalization [3]. According to the currently favored model, gp120 interacts with the extracellular regions of the coreceptor and undergoes a conformational change that releases constraints on gp41, thereby unleashing its fusogenic activity.

The gp120 Interaction with Coreceptors: A Challenging Structural Problem

Despite extensive studies aimed at defining the precise molecular choreography of the gp120-coreceptor pas de deux, the details remain obscure [15]. Particular importance has been assigned to the N-terminal region and the 2nd extracellular loop (ECL) of CCR5. A major obstacle is the extraordinary difficulty of solving coreceptor structure at the atomic level; indeed to date, a 3-dimensional X-ray crystallographic structure has been obtained for only one GPCR: bovine rhodopsin [5, 16]. With high resolution X-ray structures solved for gp120 in its CD4-bound [17] and unbound [18] forms, the post-fusion core of gp41 [19,



B.

Code	Peptide	CCR5 region	Amino acid sequence
PNt	1 - 26	N-terminus	MDYQVSSPIYDINYYTSEPCQKINVK
PL1	90 - 101	ECL1	AAAQWDFGNTMC
PL2	168 - 191	ECL2	RSQKEGLHYTCSSHFPYSQYQFWK
PL3	261 - 276	ECL3	QEFFGLNNCSSSNRLD

Fig. 1. Panel A: Schematic diagram of CCR5 [23]; the shaded residues correspond to the synthetic peptides analyzed. Panel B: Peptide sequences.

20], and the gp120-binding region of CD4 [21, 22], the coreceptor structure is conspicuous for its absence from the fusion ballet troupe. In this review, we describe insights gained from studies with synthetic peptides derived from the extracellular regions of CCR5, including the N-terminal segment and the three extracellular loops (Fig. 1).

Peptides Based on the CCR5 N-terminal Region

The amino terminal segment of CCR5 consists of roughly 30 amino acids, including a stretch prior to the cysteine residue at position 20 that is acidic and tyrosine-rich (Fig. 1). This region, and particularly the tyrosines and acidic residues, were shown by mutagenesis studies to be critical for both HIV-1 entry and binding of gp120-CD4 complexes [24-27]. It was subsequently found that at least two of the four the N-terminal tyrosine residues are sulfated, and that this post-translational modification is important for coreceptor activity [28]. Interestingly, it was noted that other GPCRs known to display coreceptor activity for HIV and the related simian immunodeficiency virus also share the feature of tyrosine-rich and acidic N-termini (though they share little sequence homology in the external regions).

Synthetic peptides derived from the CCR5 N-terminus provided new insights into the importance of tyrosine sulfation [29-31]. Analyses of peptide binding to a CD4-enhanced determinant on gp120, peptide inhibition of gp120-CD4 complex binding to CCR5, and peptide blockade of HIV-1 entry confirmed the importance of tyrosine sulfation, particularly at positions 10 and 14. The activities were selective for Envs from CCR5-using (particularly CCR5-specific) variants, and were not observed with peptides containing the corresponding phospho-tyrosine substitutions. Results suggested that the CCR5 tyrosine-sulfated N-terminal peptides interact with determinants in both the stem of the V3 loop and the conserved C4 region of gp120 [30-32]. Interestingly, these peptides were able to functionally reconstitute the coreceptor activity of a N-terminal truncated CCR5 construct [33].

Taken together, these results highlight the critical importance of the N-terminus of CCR5 in its coreceptor function, with particular focus on sulfated tyrosine residues in this region. However the findings also supported the idea that other portions of CCR5 are involved, a conclusion that was solidified by the isolation of HIV-1 variants adapted to use a CCR5 construct lacking the sulfated N-termnal region [34, 35]. As described in the following section, CCR5-based synthetic peptides have provided further insights.

Peptides Based on the CCR5 Extracellular Loops

Extensive molecular biological and immunological studies have implicated the CCR5 ECLs in coreceptor function; the importance of ECL-2 has been particularly highlighted [15]. We demonstrated that peptides derived from each of the ECLs (see Fig. 1) caused dose-dependent inhibition of HIV-1 Env-mediated fusion and virus infection. The inhibitory effects varied in terms of their potencies and specificities for different HIV Envs. For example, assays of Env-mediated cell fusion revealed potent inhibition by the ECL-2 peptide for the macrophage tropic (R5) Ba-L Env, but little effect on the dual-tropic (R5X4) 89.6 Env; this distinction was observed with varying target cell types, e.g. mouse fibroblasts expressing recombinant human CD4 plus CCR5 (Fig. 2) or activated human PBMCs. By contrast, the ECL-1 and ECL-3 peptides had com-

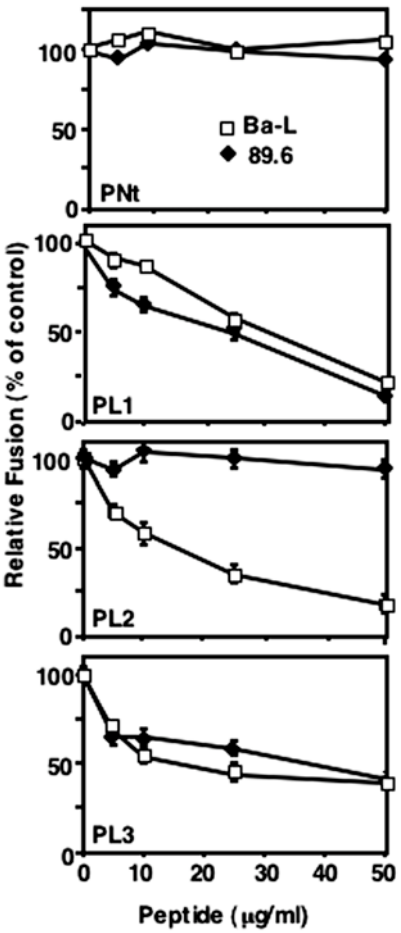


Fig. 2. *Inhibition of cell fusion by peptides based on the* ECLs of CCR5 [23]. Fusion between effector cells expressing the indicated Env and NIH-3T3 cells expressing recombinant human CD4 + CCR5 was quantitated by a reporter gene assay measuring β-galactosidase production in fused cells [36].

parable inhibitory activities against fusion mediated by either the Ba-L or 89.6 Envs. As expected, the CCR5 N-terminal peptide lacking sulfated tyrosines had no effect. Parallel results were obtained in assays of productive infection. None of the CCR5 ECL peptides inhibited fusion or infection mediated by the CXCR4-specific (X4) LAV Env.

Several lines of evidence indicated that the inhibitory effects of the ECL peptides were a consequence of their interactions with the HIV-1 Env: (a) the ECL-2 inhibitory activity persisted after pretreating and washing the Env-expressing effector cells, but not the receptor-expressing target cells; (b) the potency of ECL-2 fusion inhibition correlated inversely with the expression level of Env, but not of CCR5; (c) for a given Env, the ECL-1 peptide sensitivity was comparable when different coreceptors were used; and (d) the ECL-2 peptide inhibited CCR5 binding of sCD4-activated radio-iodinated gp120 from the JR-FL (R5) strain, but not the 89.6 (R5X4) strain.

The simplest interpretation of these results is that the ECL peptides associated with the specific regions of gp120 that normally interact with the corresponding CCR5 domains, thereby blocking the gp120/CCR5 interactions required for fusion. These findings provide additional support for the notion that Envs from different HIV-1 strains interact with CCR5 in varying ways. This notion is compatible with findings that HIV-1 variants selected for resistance to a low-MW CCR5 blocking agent had adapted to use the drug-bound form of the coreceptor [37].

Future Directions

The findings described herein indicate that synthetic peptides derived from the extracellular regions of CCR5 represent valuable probes for structure-function analyses of gp120-coreceptor interactions. The emerging picture is that CD4-activated gp120 interacts with a molecular surface displayed on extracellular portion of CCR5, and can evolve to function in varied ways with this or alternate coreceptors. Thus rather than a "lock-in-key" mechanism, the HIV coreceptor interaction behaves like a slippery slope, with many experimental pathways to follow and pitfalls to avoid in the coming years.

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Curriculum Vitae

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Edward Berger obtained his B.S. in Chemistry in 1968 from City College of the City University of New York, and then his Ph.D. in Biochemistry and Molecular Biology in 1973 from Cornell University, Ithaca, NY. He completed postdoctoral fellowships, from 1973-1976 at Stanford University School of Medicine, Stanford, CA, and from 1976-1977 at Scripps Clinic and Research Foundation, La Jolla, CA. He then joined the faculty at the Worcester Foundation for Experimental Biology, Shrewsbury, MA, where he served as Staff Scientist in the Cell Biology Group from 1977-1987. In 1987 he joined the Laboratory of Viral Diseases at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, and since 1995 has served as Chief of the Molecular Structure Section. The major focus of his research is on the mechanisms of virus entry, and the development of novel antiviral strategies based on molecules involved in entry. His major basic contributions include the first discovery of HIV coreceptors (CXCR4, followed by CCR5), and the recent identification of an entry receptor for KSHV. He has also developed novel antiviral approaches against HIV, including immunotoxins to deplete infected cell reservoirs and a potent bifunctional neutralizing protein based on the sequential receptor interaction model of entry.

Curriculum Vitae

Ghalib Alkhatib

Ghalib Alkhatib obtained his B.Sc. in Biology from Pahlavi University, Shiraz, Iran. He was a graduate student in Montreal, Quebec, Canada, where he received his Ph.D. from McGill University in 1989. He was trained in Molecular Immunology at the Mount Sinai Hospital Research Institute, Toronto, Canada. He was a visiting fellow in HIV/AIDS in the research group of Edward Berger, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA. His work there led to the identification of CCR5 as a coreceptor for macrophage-tropic HIV-1 in 1996. In 1997 he was appointed Assistant Professor in the Department of Microbiology and Immunology at the Indiana University School of Medicine in Indianapolis, IN. He is currently Associate Professor and Assistant Member of the Walther Oncology Center in the Indiana University School of Medicine.

The major focus of his current research is investigating the mechanism of resistance to HIV-1 infection by individuals lacking CCR5 expression. His laboratory is also actively involved in studying the mechanism of antiviral activity of the newly discovered CXCL12 isoforms.
