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Therapeutic Approaches to Human Immunodeficiency Virus: Structural Studies on G-Protein-Coupled Receptors

Garland R. Marshall

DEPARTMENT OF MOLECULAR BIOLOGY AND PHARMACOLOGY AND THE CENTER FOR MOLECULAR DESIGN,
WASHINGTON UNIVERSITY MEDICAL SCHOOL, ST. LOUIS, MO 63110, USA

ABSTRACT. Infection by human immunodeficiency virus (HIV) requires the presence of a chemokine receptor on the susceptible cell. The expression of two different chemokine receptors on macrophages and lymphocytes explains the selectivity of different HIV isolates. The rationale behind the choice of the chemokine receptor (CCR5) expressed on macrophages as a therapeutic target is based on the epidemiological studies of the impact on HIV infectivity of a human mutation that prevents expression of this receptor. CCR5 is a member of the G-protein-coupled receptor family, which has yet to be characterized structurally at atomic resolution. Efforts to model the three-dimensional structure of such receptors and to characterize them experimentally are underway in many laboratories. As an example, structural studies determining the bound conformation of the C-terminal peptide of the α -subunit of transducin, the relevant G-protein of vision, with rhodopsin are presented. PHARMACOL. THER. 76(1-3):135-139, 1997. © 1997 Elsevier Science Inc.

KEYWORDS. HIV, chemokine receptor, NMR, transducin, rhodopsin, GPCR.

CONTENTS

1. INTRODUCTION	135	LIGAND INTERACTIONS	136
2. HUMAN IMMUNODEFICIENCY VIRUS CO-RECEPTORS	135	4. CONCLUSIONS	138
3. STRUCTURAL APPROACHES TO G-PROTEIN-COUPLED RECEPTOR-		ACKNOWLEDGEMENTS	138
		REFERENCES	138

ABBREVIATIONS. AIDS, acquired immunodeficiency syndrome; GPCR, G-protein-coupled receptor; Gt, transducin; HIV, human immunodeficiency virus; Rh^{*}, light-activated rhodopsin; TRNOE, transferred nuclear Overhauser effect.

1. INTRODUCTION

Numerous therapeutic approaches to human immunodeficiency virus (HIV) have been explored. One of the major impediments to effective HIV therapy is the rapid development of resistance against either reverse-transcriptase or HIV-protease inhibitors (Erickson and Burt, 1996; Moutouh *et al.*, 1996). Only cocktails of combinations of inhibitors have given hope of long-term treatment and possibly a cure for acquired immunodeficiency syndrome (AIDS). Such cocktails, however, are very expensive, as each drug must be given at a sufficiently high dose to suppress any viral mutation that would cause resistance to the other components of the cocktail. Current combination therapy employs a mixture of a reverse-transcriptase inhibitor with the recently developed HIV protease inhibitors. The development of HIV protease inhibitors has been a testbed for application and development of structure-based drug design methodology. This effort was stimulated by the determination of the three-dimensional structure of the first complex of HIV protease with an inhibitor, MVT-101 (Miller *et al.*, 1989). The inhibitor was synthesized in my laboratory by Mihaly V. Toth, the HIV protease was prepared by solid phase synthesis in the laboratory of Stephen Kent at Caltech (Pasadena, CA, USA), and the structure of the complex was determined by Maria Miller in the group of

Alex Wlodower at the National Cancer Institute (Frederick, MD, USA). The immediate availability of this structure to the scientific community stimulated rapid evolution of many families of HIV protease inhibitors, with three currently being available in the clinic and several more in the final stages of clinical evaluation. The major difficulty with the clinical utilization of combination therapy with these compounds is the cost, estimated at \$20,000 (US) per year. This makes their application economically impossible in Third World countries such as India, where the per capita income is less than \$400 (US) per year. Clearly, vaccines are the logical approach to global HIV control, but many logistical problems remain to be solved. One major problem facing vaccines, as well as therapeutics based on viral targets, is the rapid mutation rate of viral transcription that quickly leads to resistance.

2. HUMAN IMMUNODEFICIENCY VIRUS CO-RECEPTORS

In the last year, other attractive targets for an HIV therapeutic have emerged in the literature (Fauci, 1996). While the interaction of gp120 on the surface of the virus and CD4 on the surface of the cellular host is necessary, it is not sufficient for HIV infectivity. A co-receptor is essential on the cellular surface, and the presence of different co-recep-

tors explains the tropism of a strain of HIV isolates for macrophages and lymphocytes (Alkhatib *et al.*, 1996; Bates, 1996; Berson *et al.*, 1996; Choe *et al.*, 1996; Combadiere *et al.*, 1996; Deng *et al.*, 1996; Dragic *et al.*, 1996; Friedland, 1996; Trkola *et al.*, 1996; He *et al.*, 1997). The macrophage expresses the chemokine receptor CRC5, while the lymphocyte expresses CXCR4, on its surface. HIV strains selective for macrophages have a different sequence in the V3 loop of gp120 than those selective for lymphocytes (Hwang *et al.*, 1991), suggesting a direct interaction between the chemokine receptors and the V3 loop during a critical phase of infectivity. Mapping of the chemokine receptors with chimeric receptors and mutational analysis to determine those areas responsible for the co-receptor activity is underway. Blocking the interaction of gp120 with chemokine receptors (Simmons *et al.*, 1997) may well have useful therapeutic benefits, especially if one can target the mammalian chemokine receptor itself, which does not mutate with the frequency of a virally encoded protein. One question impeding such an approach is the possible functional necessity for the chemokine receptor in normal physiology and pathophysiological states other than AIDS.

An existing mutation in the human population, in effect, already has done the "knock-out" experiment (Dean *et al.*, 1996; Samson *et al.*, 1996). Analysis of the chemokine receptors of individuals who have been HIV positive for many years without developing AIDS have shown that they are heterozygous for a defective chemokine receptor CKR5D32, which is nonfunctional. Individuals who are homozygous for this receptor mutant are HIV negative, even though they have been chronically exposed. Cells from homozygous individuals do not have CCR5 expressed on their surface and are unresponsive to MIP-1, one of the natural chemokines. Thus, blockade of the chemokine receptor and prevention of complex formation with the gp120-CD4 complex is a very plausible therapeutic objective, a fact that has not gone unnoticed by the pharmaceutical industry (Cohen, 1997). In order to effectively employ structure-based design techniques in developing such a therapeutic, structural information on the complex between the V3 loop of gp120 and the chemokine receptor is essential. Unfortunately, the chemokine receptors are seven transmembrane or G-protein-coupled receptors (GPCRs), which have resisted all efforts to generate high-resolution crystal structures.

3. STRUCTURAL APPROACHES TO G-PROTEIN-COUPLED RECEPTOR-LIGAND INTERACTIONS

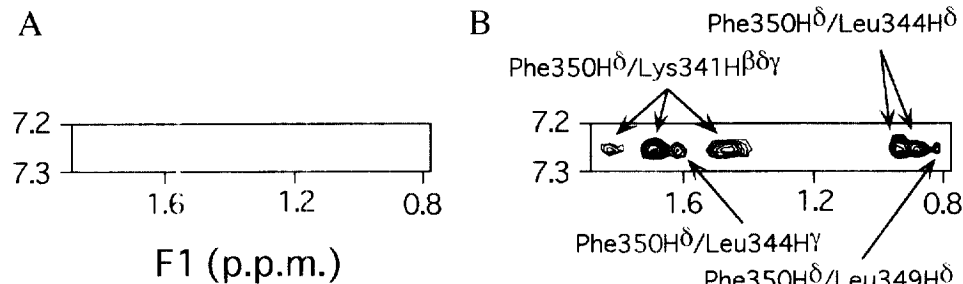
An experimental paradigm for the direct determination of the conformation of the V3 loop when bound to the GPCR CCR5 would be most welcome. A significant problem facing such an experimental approach is the current limitation of CCR5 receptor availability. In order to prototype such an experimental approach, another GPCR was sought where adequate quantities of receptor were readily available. Visual signals in retinal rod cells are triggered by the interac-

tion of photoexcited rhodopsin (Rh*) with the heterotrimeric GTP-binding protein transducin (Gt). This GPCR has been well characterized with regard to light activation, and is readily available in quantities sufficient for biophysical studies from bovine retina. Molecular details of the mechanism by which Rh* recognizes Gt and catalyzes nucleotide exchange remain obscure. Several domains on the α -, and $\beta\gamma$ -subunits of Gt contribute to its Rh* binding site and an 11-residue segment, IKENLKDCGLF, at the carboxyl terminus of the α -subunit, Gt α (340–350), has been shown to play a major role in receptor recognition and nucleotide exchange (Hamm *et al.*, 1988; Garcia *et al.*, 1995; Osawa and Weiss, 1995). This C-terminal segment of Gt α is disordered in all X-ray structures of Gt (Noel *et al.*, 1993; Lambright *et al.*, 1994; Sondek *et al.*, 1994; Lambright *et al.*, 1996). Transferred nuclear Overhauser effect (TRNOE) spectroscopy was used to elucidate the structure of the Gt α (340–350) peptide when bound to Rh* (O. Kisselev *et al.*, submitted for publication). Despite the fact that this peptide binds to the intracellular face of Rh* rather than to the external face, as does the V3 loop to CCR5, the success of this approach provides a model for the direct experimental determination of the CCR5-bound conformation of the V3 loop.

In the dark-adapted state of Rh*, only two medium-range NOE interactions were observed in the sample of Gt α (340–350) with Rh*. A remarkable increase to 98 from 2 in the number of medium- and longer-range interactions in the peptide was observed on exposure to 495 nm light, indicative of a discrete bound conformation to light-activated Rh*. The peptide conformation in the Rh*-bound state gave a total of 103 additional NOE cross-peaks, such as those seen in the aromatic region (Fig. 1). These additional NOE cross-peaks relax in parallel to the decay of the light-activated form of Rh*, metarhodopsin II, thought to bind and activate Gt. This argues for specific binding of Gt α (340–350) to Rh* and parallels the ability of Gt to form a transient high-affinity complex with Rh* (Bornancin *et al.*, 1989). The paucity of medium- to long-range NOEs in the dark-adapted spectra indicates minimal, if any, binding of Gt α (340–350) to dark-adapted Rh*.

Independent computations of the NMR structure of Gt α (340–350) bound to Rh* gave 20 structures with a high degree of convergence ($0.30 \pm 0.12 \text{ \AA}$ r.m.s.d. for C α s), with a helical turn in the middle of the undecapeptide followed by an open reverse turn (Fig. 2). The Rh*-bound structure of Gt α (340–350) is characterized by multiple strong peaks in the TRNOE spectra between the Phe350 aromatic ring and the side chains of Leu349, Leu344 and Lys341 (Fig. 1). This hydrophobic cluster favors formation of a hydrogen bond between the carbonyl oxygen of Leu344 and the amide hydrogen of Gly348, which additionally stabilizes the reverse turn. The helical turn involving Glu342, Asn343, Leu344, Lys345, and Asp346 is supported by 48 medium- and long-range NOEs, and 2 main-chain hydrogen bonds between Lys341–Lys345 and Glu342–Asp346. The NOE cross-peak between the amide protons of Lys345

FIGURE 1. Cross-peaks in the aromatic-aliphatic region showing interactions between F350 aromatic protons and side chain protons of L349, L344, and K341 from the NOESY spectra of G α (340–350) in the presence of the dark-adapted (left panel) and photoexcited Rh* (right panel).



and Asn343, (NH_{i,i+2}) agrees with the presence of a helical turn. Gly348 is highly conserved in G-proteins of Gt and Gi families, and appears invariant at position 348 in 24 G α (340–350) peptide analogs selected for Rh* binding (Martin *et al.*, 1996), consistent with the ϕ/ψ torsional angles of Rh*-bound G α (340–350). Expression of heterotrimeric Gt mutants with substitutions of Gly348 by Ala or Pro (Garcia *et al.*, 1995; Osawa and Weiss, 1995) in the α -subunit severely impairs interaction with Rh*. Based on earlier NMR studies, Gly348 was suggested to be part of a Type II β -turn at the C-terminus of the α -subunit (Dratz *et al.*, 1993), which our results do not support. Comparison of the Rh*-bound structure of G α (340–350) with known types of helix-terminating motifs revealed a high degree of similarity with the α L type of helix capping characterized by a hydrogen bond between the amide hydrogen at C' and carbonyl oxygen at C3 (5 \rightarrow 1 type). When docked to the GDP-liganded crystal structure of Gt, the NMR structure of G α (340–350) provides a perfect continuation of the C-terminal helix α 5, G α (325–340), terminated by the C-cap and hydrophobic cluster. The hydrophobic patch (Lys341/Leu344/Leu349/Phe350) points in the direction where Rh* would be found in a putative ternary complex with Gt. Hydrophobic shielding of this motif from water clearly requires a specific binding site on Rh*. Light-induced exposure of a potential complementary binding site on the second (CD) and third (EF) intracellular loops (Acharya *et al.*, 1997) is consistent with the movement of transmembrane helices C and F recently shown to be required for light activation (Farrens *et al.*, 1996; Sheikh *et al.*, 1996). Dissociation from the receptor

would be expected to expose Leu349 to solvent, leading to transition of the G α (340–350) segment back into a disordered state. The pivotal roles of Leu344 and Leu349 in Rh* binding have been established with specific mutations at these sites in Gt and by studies of G α (340–350) peptide analogs.

Several different considerations contribute to the credibility of our results. The hydrophobic cluster on the exterior of the peptide is unexpected in water; the cation-aromatic interaction between the Lys341 ϵ -amine and the Phe350 phenyl ring is commonly seen in the interior of proteins and is often exploited in specific ligand-receptor recognition (Dougherty, 1996). Helical turns at the terminals of small peptides in solution are also somewhat unusual. Also, similar TRNOE experiments on an analog of G α (340–350) with an affinity approximately 100-fold greater show diminished NOE peaks in accord with the requirements for an appropriate exchange rate in ligand-receptor complexes. TRNOE experiments can produce artifacts due to spin diffusion via receptor protons, and solids CPMAS NMR experiments of specifically labelled analogs of G α (340–350) are in progress to confirm and refine the Rh*-bound conformation. Our results provide direct evidence for the reversible ordering of the C-terminal 11-residue segment of G α induced by Rh*. The C-terminus of G γ is also known (15–17) to interact with Rh*, and the combination of interactions could affect the interface between G α and G $\beta\gamma$. If structurally coupled to the conformation of the nucleotide binding site, Rh*-catalyzed C-capping of G α (340–350) could trigger GDP release and subsequent GTP binding, resulting in second messenger production.

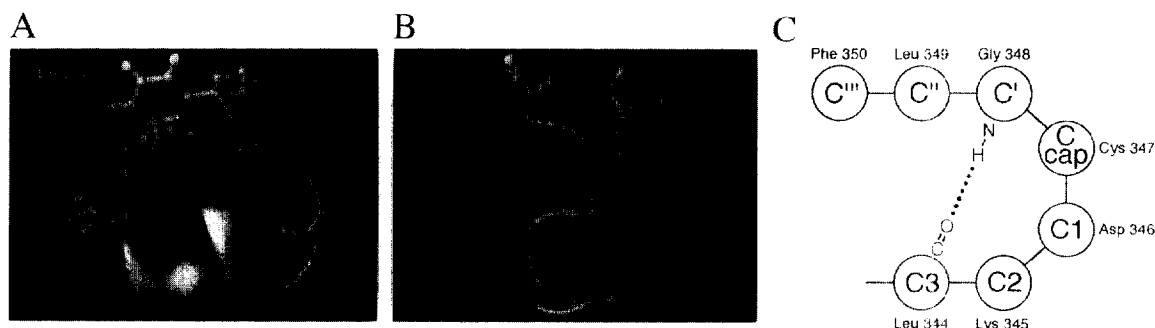


FIGURE 2. Two orthogonal views of Rh*-bound conformation of G α (340–350) IKENLKDCGLF as determined by TRNOESY. A, front; B, end view; C, scheme of the C-cap motif.

4. CONCLUSIONS

The feasibility of an experimental strategy for determination of the receptor-bound conformation of biologically active peptides binding to the extracellular loops of GPCRs, including determination of the bound conformation of the V3 loop to CRC5, has been demonstrated. In order to implement this strategy, sufficient quantities of the CRC5 receptor will have to be cloned, expressed, purified, and reconstituted. Logistically, this is a significant undertaking, which could consume several years of research. The CCR5-bound structure of the V3 loop would provide, however, a structural basis for the design of novel therapeutics that could inhibit HIV infectivity by blocking the interaction of gp120 and its chemokine co-receptor. Such compounds would have a potential prophylactic use, as well as a use in the therapy of HIV-positive patients, and would provide a novel approach to HIV therapeutics.

Alternatively, screening of compound libraries for leads that inhibit the interaction of the V3 loop with CCR5 already are underway in many laboratories. There is evidence that suggests that a class of HIV inhibitors, the bicyclams (Bridger *et al.*, 1995, 1996), detected through screening may interfere with this interaction, based on the fact that resistance to these compounds in cultured cells was shown to map to mutations in the V3 loop (De Vreese *et al.*, 1996a,b). Bicyclam-resistant virus showed cross-resistance to sulfated polysaccharides, such as dextran sulfate, pentosan sulfate, heparin and cyclodextrin sulfate, suggesting that these compounds may share a common mechanism of action. A naphthalenesulfonic acid derivative FP-21399, whose parent was isolated by screening, has been shown to competitively inhibit interactions between the V3 loop and HIV co-receptors (Ono *et al.*, 1997). Experiments to determine if bicyclams directly prevent V3 loop-CCR5 interaction are currently underway (G. R. Marshall and L. Ratner, unpublished).

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