Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1768 (2007) 2107–2119
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbamem

Prediction of HIV-1 entry inhibitors neomycin–arginine conjugates interaction with the CD4-gp120 binding site by molecular modeling and multistep docking procedure

Alexander Berchanski, Aviva Lapidot *

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, 76100, Israel

Received 28 November 2006; received in revised form 4 April 2007; accepted 19 April 2007
Available online 1 May 2007

Abstract

Developing of multi-target HIV-1 entry inhibitors represents an important avenue of drug therapy. Two such inhibitors are hexa-arginine-neomycin-conjugate (NeoR6) and nona-D-arginine-neomycin-conjugate (Neo-r9). Our findings that NeoR6-resistant mutations appear in the gp120 constant regions; and NeoR6 is not CCR5 antagonist, but inhibits CXCR4 and CCR5 HIV-1 using isolates, led us to suggest that NeoR6 may inhibit HIV-1 entry by interfering with the CD4-gp120 binding. To support this notion, we constructed a homology model of unliganded HIV-1_{IIIB} gp120 and docked NeoR6 and Neo-r9 to it, using a multistep docking procedure: geometric–electrostatic docking by MolFit; flexible ligand docking by Autodock3 and final refinement of the obtained complexes by Discover3. Binding free energies were calculated by MM-PBSA methodology. The model predicts competitive inhibition of CD4-gp120 binding by NeoR6 and Neo-r9. We determined plausible binding sites between constructed CD4-bound gp120 trimer and homology modeled membranal CXCR4, and tested NeoR6 and Neo-r9 interfering with this interaction. These models support our notion that another mechanism of anti-HIV-1 activity of NeoR6 is inhibition of gp120-CXCR4 binding. These structural models and interaction of NeoR6 and Neo-r9 with gp120 and CXCR4 provide a powerful approach for structural based drug design for selective targeting of HIV-1 entry and/or for inhibition of other retroviruses with similar mechanism of entry.

Keywords: Hexa-arginine-neomycin-conjugate (NeoR6); poly-arginine-neomycin-conjugate (Neo-r9); Molecular modeling and docking; HIV-1 attachment inhibitor; HIV-1 gp120; CD4/CXCR4

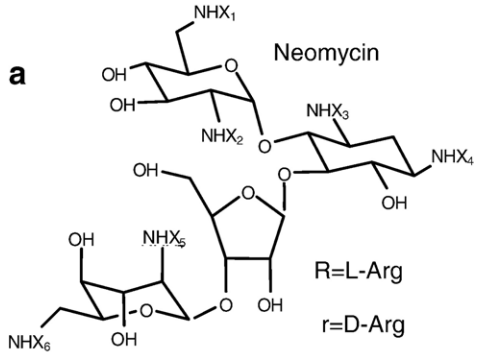
1. Introduction

Infection with the human immunodeficiency virus (HIV) starts with the virus particle attachment to the cell surface followed by viral membrane fusion with the target cell membrane, thereby allowing entry of the viral genetic material into the cytosol [1,2]. The binding of the trimeric envelope glycoprotein gp120–gp41 to the cell surface receptor CD4 and chemokine coreceptors CXCR4 or CCR5 triggers a series of conformational changes in the envelope proteins. While performing distinct operations in HIV-1 entry, the activities of the gp120 and gp41 protomers must be highly coordinated in order to lead to successful infection (reviewed in [1,2]). Binding is enabled via the interaction of the most N-terminal of the immunoglobulin-like domains of CD4 with a cavity in gp120 [3]. CD4 contacts all three gp120 core domains and is thought to bring the inner and outer domains into proximity and to structure the conformationally labile bridging sheet. Many of the important contacts between gp120 and CD4 occur at the interface of the three gp120 core domains. The binding of gp120 and CD4 creates a roughly spherical cavity at this location. This cavity extends deep into the interior of gp120 and is bound by residues from each of the gp120 core domains. These cavity-lining gp120 residues are highly conserved among HIV-1 isolates. Thus, CD4-binding cavity has been suggested as a potential target for drug design [3,4]. The compounds that prevent HIV-1 gp120 binding to CD4 are called attachment inhibitors.

Previously our group has designed and synthesized a set of novel peptidomimetic substances, conjugates of aminoglycoside antibiotics with arginine [1,5–9]. The aminoglycoside-arginine

* Corresponding author. Tel.: +972 8 9343413; fax: +972 8 9344142.
E-mail address: aviva.lapidot@weizmann.ac.il (A. Lapidot).

0005-2736/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2007.04.017



Compound	X_1	X_2	X_3	X_4	X_5	X_6
Neomycin B	H	H	H	H	H	H
NeoR6	R	R	R	R	R	R
Neo-r9	r9-NHAc	H	H	H	H	H

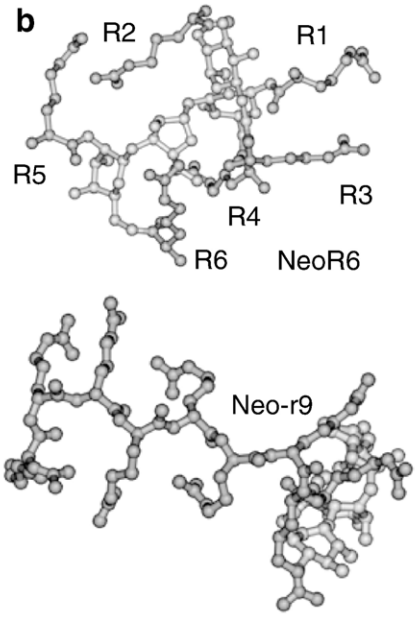


Fig. 1. (a) NeoR6 and Neo-r9. (b) NeoR6 and Neo-r9 in the bound conformation with CXCR4.

conjugates (AACs) represent a new class of compounds that may serve as lead compounds for the development of multi-target HIV-1 inhibitors [1]. The AACs, efficiently penetrate cells, including neurons, and accumulate intracellularly [5–7,10]. The AACs inhibit HIV-1 infection and proliferation in cultured human lymphocytes, displaying low cytotoxicity [5,7,11], inhibit gp120-triggered death in human neuroblastoma cells, and cross the blood brain barrier [11]. Neomycin–arginine conjugate (NeoR6, Fig. 1), designed as Tat-mimetic, functions also as HIV-1 entry inhibitor by interacting with CXCR4 [7,11–13] at the binding site of the anti-CXCR4 mAb 12G5 [7]. The interaction between HIV-1 and CXCR4 involves the variable 3 (V3) loop of gp120 [14]. Since NeoR6 is a V3 mimetic, comprising six arginine moieties, it may also compete with the gp120 V3 loop for CXCR4 receptor binding and thus inhibit entry of HIV-1 into cells. The crystal structure of V3 loop in the context of an HIV-1 gp120 core complexed to the CD4 receptor and to the X5 antibody was recently determined [15]. These structures provide a structural rationale for the role of V3 loop in HIV-1 entry and evasion of the immune system [15]. Of note, we found in NeoR6 resistant ($NeoR6^{res}$) viral isolates mutations in the constant regions C3 and C4, and in the variable region V4 of gp120 and 2 mutations in the heptad repeat 2 (HR2) of gp41 [16]. Importantly, two of the three gp120 mutations (I341T and Q398K) are located in the CD4-binding cavity of gp120. These results led us to suggest that NeoR6 may also inhibit HIV-1 entry by interfering with the gp120-CD4 binding and with the pre-hairpin intermediate of the fusion process [16].

Recently, we synthesized a novel set of D- and L-poly-arginine aminoglycoside conjugates (pAACs) and their antiviral activities are currently being investigated [8]. Our findings indicate that the most potent pAAC, nona-D-arginine-neomycin-conjugate (Neo-r9, Fig. 1) inhibits a variety of T-tropic HIV-1 isolates, including $NeoR6^{res}$ isolates. Since Neo-r9 has a relatively similar structure to NeoR6, we suggested that, as well as NeoR6, it may also inhibit HIV-1 entry by interfering with CD4-gp120 and gp120-CXCR4 bindings. Moreover, because Neo-r9 is significantly more positively charged than NeoR6, we predict that it can interact also with the gp120 residues of $NeoR6^{res}$ strains.

There are a number of drugs whose development was heavily influenced by or based on structure-based design and screening strategies, such as HIV-1 protease inhibitors [17]. Computational methodologies have become a crucial component of many drug discovery programs, from hit identification to lead optimization, approaches such as ligand- or structure-based virtual screening

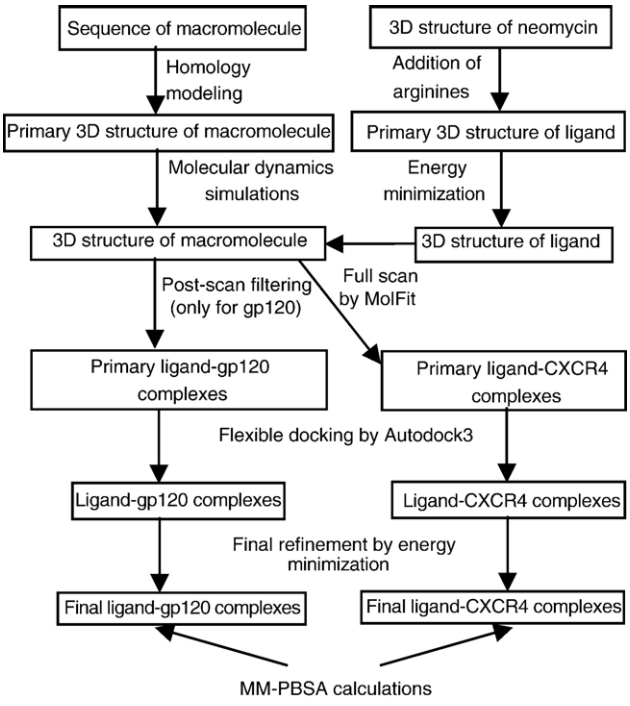


Fig. 2. Schematic representation of the molecular modeling and the multistep docking procedure followed in this study.