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Biophysical evidence of two docking sites of the carboxyl heptad repeat region within the amino heptad repeat region of gp41 of human immunodeficiency virus type 1

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Abstract

Two HIV-1 gp41-derived peptide fusion inhibitors, T-20 and T-649, were synthesized and their binding profiles of the N-heptad repeat region (HR1) were compared to examine the molecular basis of the differential antiviral potency and viral resistance. Turbidity clearance experiments based on the overlapping 15-mer peptides derived from HR1 revealed a major binding site at the LLSGIV segment for both T-20 and T-649. Additionally, another docking site was found at the sequence encompassing the hydrophobic pocket of HR1 for T-649. Concordant results were observed from the surface plasmon resonance measurements. The binding affinity profile exhibited a major maximum around the LLSGIV motif for the two peptide fusion inhibitors while a less prominent docking region was located near the hydrophobic pocket for T-649. This bi-modal model deduced from T-20 and T-649 interaction with HR1 peptides could rationalize the failure of emergence of the fusion inhibitor-resistant virus with simultaneous mutations in each of the two binding regions, as well as the generally higher potency of T-649 against most viral strains.

Keywords: Heptad repeat region; Helix bundle; Fusion inhibitor; HIV-1; gp41 hydrophobic pocket; Turbidity clearance; Surface plasmon resonance; Bi-modal binding

1. Introduction

Despite the effective combination treatment on the HIV-1 infection targeting reverse transcription and viral protease in reducing the viral titer and the progress of the disease, the emergence of resistance strains necessitated the search for antiviral agents that interfere with other steps of viral life cycle. A recent addition to the anti-HIV-1 regimen is enfuvirtide (T-20), which exerts its action by blocking fusion between the virus and the target cell (Wild et al., 1994), the first step of the viral replication cycle. T-20, a peptide corresponding to the HR2 region (aa 638–673) of the transmembrane glycoprotein gp41, potently inhibits viral infectivity of a wide range of virus variants at the nanomolar level. Since then, several peptides thought to target membrane fusion (Jiang et al., 1993; Rimsky et al., 1998), including T-649 and T-1249, have been studied. T-649 is also a 36-mer peptide composed of aa 628–663 of gp41, with 10 amino acids shift to the N-terminal direction compared to T-20. In vitro and in vivo investigations revealed that in general T-649 is more potent (Heil et al., 2004) and development of resistance phenotype is more difficult than for T-20 (Chinnadurai et al., 2005; Kilby et al., 1998; Rimsky et al., 1998).

Understanding the mechanism underlying the action of the fusion inhibitors is crucial to not only the development of future generations of entry inhibitors but also the pathway and kinetics of the virus-mediated membrane fusion, in view of the increased use of fusion inhibitors as a new class of antiviral reagents. For example, T-20 has been shown to be effective when CD4 was incubated with the cells infected with the virus (Furuta et al., 1998), suggesting that the inhibitor acted on an intermediate gp41 structure exposed by the receptor binding to gp120. Similarly, T-20 and T-649 have been used to dissect the pathway of the virus-induced fusion reaction (Melikyan et al., 2006). The emergence of T-20 resistant HIV-1 strains in which the mutations have been mapped to the HR1 domain, which sometimes were accompanied by mutations in the HR2 region (Rimsky et al., 1998; Sista et al., 2004), afforded additional opportunity to unravel the interplay between the inhibitor, the viral envelope protein and the cellular receptors. The mechanism of the drug

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resistance is also important in the design of future generations of viral therapeutics.

The initial interpretation of T-20 action is the interference of the peptide with formation of the six-helix bundle in a dominant negative manner. T-20 and T-649 have been proposed to act at the exposure of HR1 domain in gp41 triggered by the Env-CD4 complex formation but before HR1 closure upon HR1–HR2 helix bundle formation (Derdeyn et al., 2001). The emergence of T-20 resistance isolates involving mutations in the GIV (aa 547–549) motif, which was subsequently expanded to the 547–556 region (Greenberg et al., 2002; Sista et al., 2004), suggests a role of the N-terminal portion of HR1 in the HR1–HR2 interaction and the mode of T-20 action. Moreover, the mutation in the GIV tripeptide has been proposed to maintain the conformation of the stem of the Rev responsive element and hence the replication fitness of the virus (Nameki et al., 2005). These investigations demonstrated that the mechanism of T-20 and its analogs that target the intermediate structures of fusion protein is multi-faceted and suggests that its elucidation may shed light on the fusion process.

To delineate the binding site of T-20 within HR1, we have carried out a turbidity clearance study using an array of overlapping 15-mer peptide encompassing residues 526–569 (Trivedi et al., 2003). It was deduced that the LLSGIV motif constituted a critical docking site of T-20. To better characterize the interaction of HR1 and HR2, we utilized an array of overlapping peptides covering the entire HR1 to map the docking sites of T-20 and T-649 by the turbidity clearance approach and by the surface plasmon resonance (SPR) technique. The measurements on these two HR2-derived peptides corroborated our previous results (Trivedi et al., 2003) and further disclosed an additional, minor binding site at the hydrophobic pocket of HR1 (Chan et al., 1997). The latter finding can account for, at least in part, the higher potency of T-649 in comparison to T-20 for a wide range of viral strains, as well as the differential sensitivity between the variants of the GIV motif of gp41 to T-20 and T-649 (Derdeyn et al., 2001). The multiple binding mode of the HR1/HR2 association probably allows the virus to escape the fusion inhibitors with lower fitness.

2. Materials and methods

2.1. Peptide synthesis

Synthesis of the HR1 peptides, T-20 (aa 638–673) and T-649 (aa 628–663) followed the protocols described previously (Chang et al., 1999). In brief, the peptides were synthesized in an automated mode by means of Fmoc chemistry with a solid phase synthesizer (model Ranin PS3, Protein Technologies (Tucson, AZ)). The synthesized peptides are N-capped by the acetyl group and C-capped with the amino group. They were cleaved from the resin and purified by HPLC on a reverse phase C18 column. The primary sequence of peptides was ascertained by electrospray or MALDI mass spectrometry. All reagents used in this study were of analytical grade. To increase the solubility of the HR1 peptides, two lysine residues were introduced to the N-terminus of the overlapping 15-mers of HR1, which span residues 526–590 (Fig. 1).

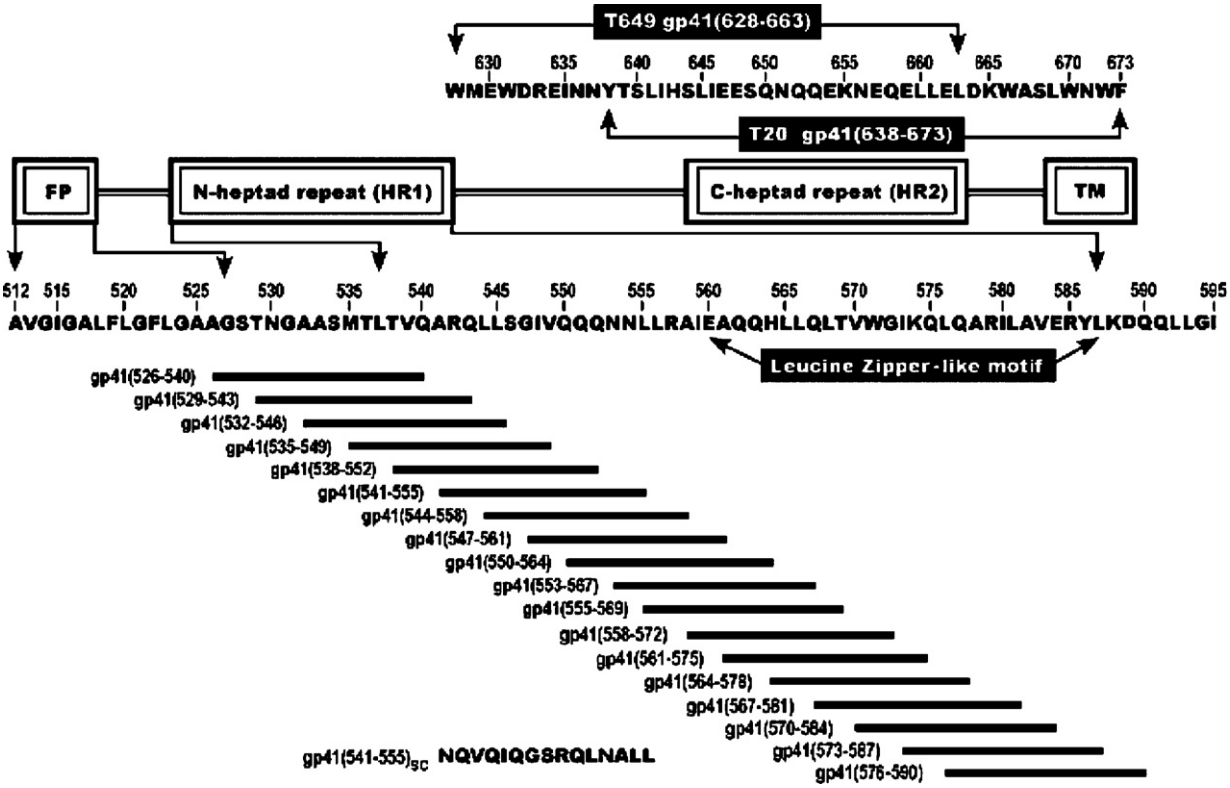

Fig. 1. The sequence of gp41 ectodomain and its functional domains. The overlapping HR1-derived peptides are indicated along with the scrambled gp41(541–555) peptide sequence. The residue numbering system is according to the HXB2 strain of HIV-1.