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A second-site suppressor significantly improves the defective phenotype imposed by mutation of an aromatic residue in the N-terminal domain of the HIV-1 capsid protein

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Abstract

The HIV-1 capsid (CA) protein plays an important role in virus assembly and infectivity. Previously, we showed that Ala substitutions in the N-terminal residues Trp23 and Phe40 cause a severely defective phenotype. In searching for mutations at these positions that result in a non-lethal phenotype, we identified one candidate, W23F. Mutant virions contained aberrant cores, but unlike W23A, also displayed some infectivity in a single-round replication assay and delayed replication kinetics in MT-4 cells. Following long-term passage in MT-4 cells, two second-site mutations were isolated. In particular, the W23F/V26I mutation partially restored the wild-type phenotype, including production of particles with conical cores and wild-type replication kinetics in MT-4 cells. A structural model is proposed to explain the suppressor phenotype. These findings describe a novel occurrence, namely suppression of a mutation in a hydrophobic residue that is critical for maintaining the structural integrity of CA and proper core assembly.
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Keywords: HIV-1 capsid protein; Second-site suppressors; HIV-1 viral cores; Dominant-negative inhibition; HIV-1 assembly; Structural models; Reverse transcriptase; Transmission electron microscopy

Introduction

The Gag polyprotein of human immunodeficiency virus type-1 (HIV-1), also known as Pr55^{gag}, is the only viral protein required for particle assembly (Freed, 1998; Vogt, 1997; Wills and Craven, 1991). During or shortly after budding, the viral protease cleaves Gag into the mature HIV-1 proteins, which include (from the N- to C-terminus): matrix; capsid (CA); nucleocapsid (NC); and p6 (Freed, 1998; Swanstrom and Wills, 1997; Vogt, 1997). Proteolytic processing of Gag induces dramatic structural rearrangements (virus "maturation"), which lead to formation of mature, infectious HIV-1 virions containing electron-dense, cone-shaped cores (Gross et al., 1998, 2000; von Schwedler et al., 1998; Wiegers et al., 1998).

The HIV-1 core has been likened to a fullerene cone (Benjamin et al., 2005; Briggs et al., 2006; Ganser et al., 1999; Li et al., 2000) and is assembled with hexameric arrays of N-terminal CA domains such that each hexamer is connected to six others by interactions with C-terminal CA dimers (Ganser et al., 1999, 2003; Ganser-Pornillos et al., 2004; Huseby et al., 2005; Lanman et al., 2003; Lanman and Prevelige, 2005; Li et al., 2000; Mayo et al., 2003). Helices I and II line the inner holes of each hexameric ring and are important for holding the rings together (Ganser-Pornillos et al., 2004; Lanman et al., 2003; Li et al., 2000). Based on the N-terminal domain structure of murine leukemia virus (MuLV) CA, it has been suggested that helix III also participates in the formation of the inner lining of

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the hexameric ring (Mortuza et al., 2004). Evidence from numerous studies supports the idea that proper assembly of conical cores is required for HIV-1 infectivity (Auerbach et al., 2006; Dorfman et al., 1994; Fitzon et al., 2000; Forshey et al., 2002; Reicin et al., 1996; Scholz et al., 2005; Tang et al., 2003a, 2001; von Schwedler et al., 1998, 2003).

The mature HIV-1 CA protein consists of 231 residues, which are folded into two independent domains connected by a short linker (Freed, 1998): an N-terminal "core" domain (residues 1–145) (Gitti et al., 1996; Momany et al., 1996; Tang et al., 2002) and a C-terminal "dimerization" domain (residues 151–231) (Gamble et al., 1997). In general, mutation of N-terminal residues does not interfere with particle production, but mutant virions often exhibit defects in reverse transcription and core assembly as well as loss of infectivity (Tang et al., 2003b and references therein; Auerbach et al., 2006; Rulli et al., 2006; Scholz et al., 2005).

In earlier work, we found that Ala substitutions at Trp23 (helix I) and Phe40 (helix II), members of a conserved group of N-terminal hydrophobic residues in HIV-1 CA (Momany et al., 1996), result in a postentry defect with the following phenotype: virions produced are noninfectious, have aberrant cores, and are unable to initiate reverse transcription in infected cells, despite the presence of a functional reverse transcriptase (RT) in virus particles (Tang et al., 2001). Additionally, mutant cores retain an excessive amount of CA and contain almost no RT (Tang et al., 2003b). In view of recent findings emphasizing the critical role of helices I and II for CA structure and our data demonstrating the specific importance of Trp23 and Phe40 for proper core assembly and overall folding of CA, we initiated a study to provide new information on the plasticity of CA, i.e., its ability to tolerate change in residues crucial for CA structure without total abrogation of biological activity. More specifically, we wanted to determine (i) whether Trp23 or Phe40 can be changed to other residues that would permit some retention of infectivity and (ii) if so, whether the virus can rescue the mutant phenotype by generating second-site suppressor mutations.

Here we report that a search for other substitutions at positions 23 and 40, which could fulfil the requirement for some viral infectivity, identified one such mutation, i.e., Trp23 to Phe. Thus, W23F exhibits a low level of infectivity in a single-round replication assay and delayed replication kinetics in MT-4 cells. Isolation of second-site suppressor mutations in CA is rare and might not occur in the case of a mutation that has a major effect on CA structure. Yet surprisingly, long-term passage of W23F in MT-4 cells led to isolation of two second-site mutations, one of which, W23F/V26I (helix I), partially restores the wild-type (WT) phenotype. A structural model that accommodates the spatial changes induced by the W23F and V26I mutations shows that hydrophobic interactions between Ile26 and Phe23 are possible and helps to explain the suppressor phenotype. The data illustrate an unusual example in which a residue so intimately involved in maintaining the structural integrity of CA can be mutated without a complete loss of functional activity and the mutation can be rescued, at least partially, by a second-site suppression mechanism.

Results

New mutations at positions 23 and 40 in the N-terminal domain of CA

In earlier studies, we showed that mutants bearing Ala substitutions for Trp23 and Phe40 (Fig. 1) have severe defects in virus replication, virion ultrastructure, and biochemical properties (Tang et al., 2001, 2003b). To further elucidate the role of these residues in maintaining CA function, we asked whether other substitutions besides Ala would result in a more moderate phenotype. A total of 13 new mutations at Trp23 (W23C, D, F, G, H, L, M, N, P, S, T, V, and Y), two aromatic substitution mutations at Phe40 (F40W and F40Y), and one double mutation, W23F/F40W, were generated. The culture supernatants from transfected HeLa cells were assayed for RT activity, and virus production was found to be ~60 to 90% of the WT value (data not shown). However, when infectivity was measured in a single-cycle assay using LuSIV cells (Roos et al., 2000), only W23F displayed any infectivity, albeit at a very low level (2% of the WT value) (see below); noninfectious mutants had ~0.5% of WT infectivity (data not shown).

The replication capacity of W23F was evaluated in long-term culture experiments by transfecting T-cell lines, i.e., H9, Jurkat, CEM (12D7), and MT-4 cells, with WT or W23F plasmid DNAs. As expected, WT virus replicated at high levels in all of these cell lines, with peak virus production reached within the first week posttransfection (Fig. 2A; data not shown). In contrast, W23F replicated *only* in MT-4 cells, but with significantly delayed replication kinetics and a small peak of virus production evident only on day 18 posttransfection (Fig. 2A; data not shown). This result could reflect the more permissive nature of MT-4 cells, which are infected with HTLV-

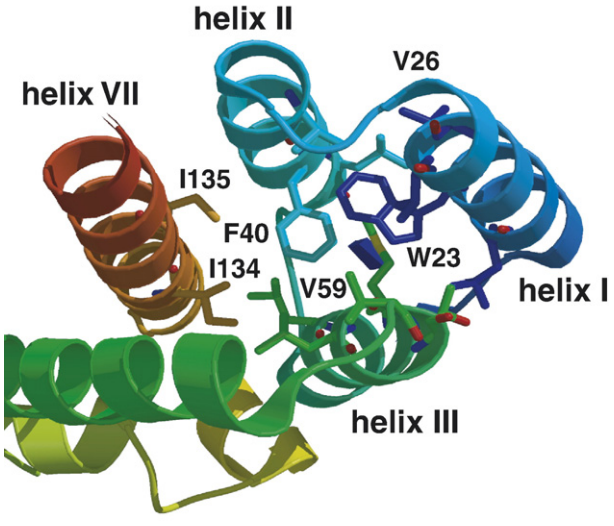

Fig. 1. Ribbon diagram highlighting helices I, II, III, and VII in the N-terminal domain of HIV-1 CA. The ribbon diagram shows a top view of helices I, II, III, and VII in the N-terminal domain structure. The positions of two conserved hydrophobic residues Trp23 (helix I) and Phe40 (helix II) are shown. The positions of Val26 (helix I), Val59 (helix III), as well as I134 and I135 (helix VII) are also illustrated in the figure.