Enhancing the Catalytic Deamination Activity of APOBEC3C Is Insufficient to Inhibit Vif-Deficient HIV-1

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http://dx.doi.org/10.1016/j.jmb.2017.03.015
Edited by Eric O. Freed

Abstract

The retroviral restriction factors of the APOBEC3 (A3) cytidine deaminase family catalyze the deamination of cytidines in single-stranded viral DNA. APOBEC3C (A3C) is a strong antiviral factor against viral infectivity factor (*vif*)-deficient simian immunodeficiency virus Δ*vif,* which is, however, a weak inhibitor against human immunodeficiency virus (HIV)-1 for reasons unknown. The precise link between the antiretroviral effect of A3C and its catalytic activity is incompletely understood. Here, we show that the S61P mutation in human A3C (A3C.S61P) boosted hypermutation in the viral genomes of simian immunodeficiency virus Δ*vif* and murine leukemia virus but not in human immunodeficiency virus HIV-1Δ*vif*. The enhanced antiviral activity of A3C.S61P correlated with enhanced *in vitro* cytidine deamination. Furthermore, the S61P mutation did not change the substrate specificity of A3C, ribonucleoprotein complex formation, self-association, Zinc coordination, or viral incorporation features. We propose that local structural changes induced by the serine-to-proline substitution are responsible for the gain of catalytic activity of A3C.S61P. Our results are a first step toward an understanding of A3C's DNA binding capacity, deamination-dependent editing, and antiviral functions at the molecular level. We conclude that the enhanced enzymatic activity of A3C is insufficient to restrict HIV-1, indicating an unknown escape mechanism of HIV-1.

Introduction

The APOBEC3 (A3) family of cytidine deaminases builds an intrinsic immune barrier against retroviruses, retrotransposons, and other viral pathogens [1–3], and recently, members of A3 were implicated in genome-wide mutation events associated with cancers [4–6] (for a review, see Ref. [7]). Humans have seven A3s (A3A–A3D, A3F–A3H). A3G, which exhibits anti HIV-1 activity, is the best-studied enzyme [8–10], and a second HIV-1 restriction factor, A3F, also displayed potent antiviral activity [11–15].

A3 enzymes mutate retroviral single-stranded DNA (ssDNA) by catalyzing the deamination of dC residues into dU. During viral assembly, A3 proteins are incorporated into progeny virions bound to viral RNA and structural proteins [16–23] and exert an antiviral effect upon subsequent infection by causing mutations in the newly reverse transcribed viral minus strand ssDNA [2]. The dC-to-dU base modification in the minus strand DNA results in dA instead of dG in plus strands, leading to coding changes and premature stop codons in the viral genome, which abort viral replication [24–26]. Additionally, deamination-independent modes may restrict the virus, for example, by inhibiting reverse transcription or interfering with integration [27–33]. As a countermeasure, lentiviruses developed the viral infectivity factor (Vif) protein that binds A3s to

target them for polyubiquitination and proteasomal degradation [34–36], thereby preventing the incorporation of A3s into budding virions. These A3–Vif interactions are often species-specific [14,37–40].

A characteristic of mammalian A3 deaminases is the presence of either one or two conserved zinc-binding (Z) domains, in which Zn^{2+} is coordinated by a Z signature (H-X-E-X_{24-31}C-X_{2-4}C, where X can be any amino acid) motif, and these domains are further classified into Z1, Z2, and Z3 domains [41–44]. A3A (Z1), A3C (Z2), and A3H (Z3) contain a single Z domain, whereas A3B (Z2-Z1), A3D (Z2-Z2), A3F (Z2-Z2), and A3G (Z2-Z1) are double Z domain proteins. All seven human A3 genes are encoded on chromosome 22, and it was speculated that duplications of single-domain genes led to the evolution of the double domain A3s [42,44]. A3C shares a high primary sequence identity of 77.4% with the C-terminal part of A3F (termed, A3F-C-terminal domain (CTD)). Of note, both A3C and A3F-CTD proteins possess the DNA deaminase catalytic function and the HIV-1 Vif binding region [14,45–48].

A3C was reported to have antiviral activity against simian immunodeficiency virus (SIV) from the African green monkey (agm), hepatitis B virus, herpes simplex virus, certain human papillomaviruses, murine leukemia virus (MLV), and HIV-1 [37,47,49–54], but there are several contradictory findings regarding its viral packaging and deamination activity [55–59]. A3C is ubiquitously expressed in lymphoid cells [60–62]. Its mRNA expression level can be stimulated by HIV-1 infection [55,57], and it was found to be significantly elevated in HIV-1 elite controllers [63]. However, A3C has no strong antiviral activity against HIV-1 lacking *vif* gene (HIV-1Δ*vif*) [9,52,55]. The moderate deamination activity of A3C on HIV-1 genomes was linked with the evolution of viral diversity rather than viral restriction [60]. Interestingly, the capacity of HIV-1 Vif to induce degradation of A3C is conserved in the majority of HIV-1 subtypes [64].

The structure of A3C, with putative DNA substrate binding pockets, was reported [46,47]. However, the biochemical aspects of A3C catalytic activity and their relevance for antiviral activity are not well explored [65]. The goal of this study was to understand the antiviral function and catalytic activity of A3C using three different viral systems. Here, we identified a single amino acid change (S61P) that increased A3C's antiviral function solely due to the enhancement of catalytic activity. We further report that the S61P substitution in A3C did not affect A3C's viral encapsidation, dimerization, nucleic acid binding, and target motif for cytosine deamination. Compared to the restriction of SIVΔ*vif* and MLV, HIV-1Δ*vif* was found to be rather resistant to this A3C mutant, suggesting an elusive escape mechanism of HIV-1Δ*vif*.

Results

Viral restriction capacity of encapsidated A3C in HIV-1Δ*vif*, SIVΔ*vif*, and MLV

To determine the antiviral activity of human A3C (hA3C), we produced luciferase reporter viruses of HIV-1Δ*vif*, SIVagmΔ*vif*, and MLV in the presence or absence of A3C, pseudotyped with the glycoprotein of vesicular stomatitis virus (VSV-G), and tested their infectivity. Figure 1a shows that hA3C inhibited SIV replication by about 2 orders of magnitude. In contrast, infectivity of MLV and HIV-1Δ*vif* virions produced in the presence of A3C was only reduced to 58% and 62%, respectively, confirming the results of other studies [9,52,55]. A3C was incorporated to similar extent into all tested viruses; thus, differential encapsidation of A3C into the viral particles cannot explain the differences in antiviral activity (Fig. 1b and c).

We then compared the impact of A3C orthologs on HIV-1 infectivity. We produced HIV-1Δ*vif* particles with A3C from chimpanzees, agm, and cat A3Z2b and with human A3F full-length or A3F-CTD (residues 184–373). Human, chimpanzee, and agm A3C proteins similarly reduced the relative infectivity of HIV-1Δ*vif* to 60%, whereas feline A3Z2b did not inhibit HIV-1Δ*vif* (Fig. 2a). The full-length A3F inhibited HIV-1Δ*vif* by nearly 2 orders of magnitude relative to the control, whereas A3F-CTD resulted in weak inhibition (Fig. 2a). In contrast to A3Cs and full-length A3F, A3F-CTD expression was low, and the packaging of A3F-CTD into HIV-1Δ*vif* was below the detection limit in our immunoblots (Fig. 2b). To possibly enhance the expression of A3F-CTD, the conserved N-terminal amino acids of A3C ^{1}MNPQI were inserted in A3F-CTD to replace ^{184}LKEIL. However, this variant also failed to form detectable levels of protein (data not shown), and hence, we used full-length A3F throughout this study.

Comparison of A3C and A3F-CTD structures

Because of its only moderate anti HIV-1 activity, deamination by A3C has not been extensively characterized [9,24,55]. To address why A3C is not very catalytically active [55], we compared the sequence and the three-dimensional structure of prototype Z2-domain protein A3C with those of the catalytically active C-terminal domain of the Z2-Z2 domain protein, A3F-CTD (Fig. 3a). The X-ray structures of hA3C and catalytic A3F-CTD were recently solved [46,66–68]. Superimposition of the structures of A3C (PDB ID: **3VOW**) and A3F-CTD (PDB ID: **4J4J**) exhibits a Cα rmsd of 0.794 Å, and the overall canonical DNA cytosine deaminase fold is intact (Fig. 3b). Given the critical roles of conserved residues in preserving the conformation