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Functional organization of human SAMHD1 and mechanisms of HIV-1 restriction

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Abstract

Sterile alpha motif and histidine-aspartate domain containing protein 1 (SAMHD1) is a triphosphohydrolase that catalyzes the conversion of deoxyribonucleoside triphosphate to deoxyribonucleoside and triphosphate. SAMHD1 has been a recent focus of study since it was identified as a potent human immunodeficiency virus-1 (HIV-1) restriction factor in the intrinsic antiviral immune system. Recent biochemical and biological studies have suggested that SAMHD1 restricts HIV-1 infection in non-cycling cells by limiting the pool of deoxyribonucleoside triphosphates, thereby interfering with HIV-1 reverse transcription. SAMHD1 also possesses single-stranded DNA and RNA binding activity, with reported nuclease activity, conferring additional HIV-1 restriction function. This review summarizes current knowledge regarding the structure of SAMHD1 and the regulation of its function in HIV-1 restriction.

Keywords

dNTPase; HIV-1; restriction factor; SAMHD1

Introduction

Intrinsic antiviral immunity is the first line of defense against retroviral infection. A primary mediator of this immunity is a subset of host cellular proteins, termed restriction factors, which play critical roles in this defense by recognizing viral components and interfering with the viral life cycle. A solid understanding of how these restriction factors defend against human immunodeficiency virus-1 (HIV-1) infection is important for the development of new therapeutic approaches to conquer the HIV-1 pandemic. In 2011, Sterile alpha motif and histidine-aspartate domain containing protein 1 (SAMHD1) was identified as an HIV-1 restriction factor, and significant progress has been made in understanding how the protein restricts post-entry HIV-1 infection. This review will focus on the recent crystal structures of SAMHD1 domains and the molecular mechanisms by which SAMHD1 activity is regulated and restricts retroviral infection.

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SAMHD1 is an HIV-1 restriction factor

The functional significance of SAMHD1 was first recognized when it was found to be associated with Aicardi-Goutières syndrome, a genetically determined neurodegenerative disorder with an autoimmunity defect (Rice et al., 2009). Two years later, the Benkirane and Skowronski groups independently discovered that SAMHD1 is an HIV-1 restriction factor (Hrecka et al., 2011; Laguette et al., 2011). Building on previous knowledge that macrophages and dendritic cells are resistant to HIV-1 infection but easily transduced by HIV-2, these two groups carried out experiments to identify the putative restriction factor that is counteracted by the viral protein x (Vpx), present in HIV-2, but not HIV-1. Using a proteomic approach, Laguette et al., identified that SAMHD1 associates with Vpx, when Vpx is stably expressed in the human monocytic THP-1 cell line (Laguette et al., 2011). Further, they found that knock-down of endogenous SAMHD1 in non-permissive THP-1 cells results in increased HIV-1 infection, while ectopic expression of SAMHD1 in SAMHD1-deficient U937 cells potently blocks HIV-1 replication. With a similar methodology, Hrecka et al., identified SAMHD1-Vpx complexes in HEK293T cells and found that Vpx mediates proteasome-dependent down-regulation of SAMHD1, by loading it onto an E3 ubiquitin ligase, cullin ring-finger ligase 4 (CRL4)-DDB1- and cullin-associated factor 1 (DCAF1) (Hrecka et al., 2011). The seminal discovery that SAMHD1 is an HIV-1 restriction factor not only answered a long standing question with regard to how HIV-2, but not HIV-1, easily transduces myeloid-lineage cells, but also opened a new field of study into how SAMHD1 functions in HIV-1 restriction. It is important to note that Vpx is a viral antagonist expressed by simian immunodeficiency virus (SIV) as well as HIV-2; several SIV strains exist and are named according to the non-human primates from which they are isolated. The Vpx expressed by each of these strains is unique in terms of sequence, and structural and functional studies of SAMHD1 have identified unique features of the Vpx-SAMHD1 interaction depending on the source/sequence of the Vpx protein (Laguette et al., 2012; Lim et al., 2012; Schwefel et al., 2014, 2015; Wu et al., 2015).

Structural and functional domains of SAMHD1

SAMHD1 comprises three regions (Figure 1A): the N-terminus (residues 1–109), a deoxyribonucleoside triphosphate triphosphohydrolase (dNTPase) catalytic core domain (residues 110–599), and the C-terminus (residues 600–626). Each of these regions has a unique structure and distinct functions, the details of which are discussed here.

The N-terminus of SAMHD1

SAMHD1 localizes to the nuclear compartment of cells owing to a nuclear localization signal sequence at residues 11–14, Lys-Arg-Pro-Arg (Brandariz-Nunez et al., 2012). SAMHD1 mutants that do not localize to the nucleus, while being competent in HIV-1 restriction, are resistant to Vpx-mediated, proteasome-dependent down-regulation. This suggests that nuclear Vpx loads SAMHD1 onto CRL4-DCAF1 E3 ubiquitin ligase for poly-ubiquitination.

The crystal structure of the mandrill monkey SAMHD1 N-terminus was solved as a ternary complex with Vpx from an SIV strain that infects mandrill monkey, and with the C-terminal

region of human DCAF1, the substrate receptor component of the CRL4 E3 ubiquitin ligase (Schwefel et al., 2015; Wu et al., 2015). In the structure, residues 1–39 form an extended loop, with an unresolved region between Ser23 and Ser33, while residues 40–109, which comprise a sterile alpha motif (SAM), fold into four α -helices (Figure 1B). Of note, the NMR solution structure (PDB code 2E8O) of the N-terminus of human SAMHD1 is essentially the same as the structure of mandrill monkey SAMHD1 observed in the crystals of the ternary complex.

The precise biological function of the SAM domain in HIV-1 restriction has not been elucidated. However, the N-terminus of SAMHD1, including the SAM domain, has undergone positive evolutionary selection, a signature of an interaction hot spot between a restriction factor and a viral antagonist (Lim et al., 2012). As a result, Vpx from SIV that infects mandrill monkey lost its ability to recruit human SAMHD1 to the CRL4-DCAF1 E3 ubiquitin ligase for proteasome-mediated down-regulation. Human and mandrill SAMHD1 have four amino acid changes in the extended N-terminal loop region and nine amino acid variations in the SAM domain. Of these, residue Phe15 in the N-terminal loop and residue Phe50 in the SAM domain of mandrill SAMHD1 make critical contacts with Vpx from SIV that infects mandrill monkey (Schwefel et al., 2015; Wu et al., 2015). Mutation of these residues significantly affect species specificity in viral antagonism. Therefore, the ternary complex structures provide mechanistic and structural insight into how Vpx proteins engage SAMHD1 and adapt to sequence variation of the host cellular target.

Catalytic core domain

The structure of the catalytic core domain (residues 110–599) has been extensively investigated over the past 5 years. The first crystal structure of the domain (residues 120–626) was determined as a dimer (Goldstone et al., 2011). In that structure, the C-terminal region (residues 584–626) was not resolved. Furthermore, a proposed dGTP allosteric activator was not present in the structure. Biochemical characterization of SAMHD1 dNTPase activity revealed that inactive SAMHD1 exists in a monomer and dimer equilibrium and undergoes dGTP-induced tetramerization to generate the catalytically active form (Yan et al., 2013). Further, the stability of tetramer was found to be closely related to the HIV-1 restriction activity of SAMHD1, and the stability was attributed to the C-terminus, located outside of the catalytic core domain. In monocytic cells, where SAMHD1 potently restricts HIV-1 infection, it predominantly exists as a tetramer (Yan et al., 2013). The structural basis of dGTP-induced SAMHD1 tetramerization was then revealed when the structure of the core (residues 110–599) was independently solved by two groups (Ji et al., 2013; Zhu et al., 2013). In the tetramer structures, two adjacent dGTP binding sites, termed primary and secondary allosteric sites, are formed by three monomers, providing multiple hydrogen bonding interactions to dGTPs (Figures 1C and 2A). Several residues, absent in the dimer structure, engage dGTP in the binding pockets at the allosteric sites, thereby stabilizing the tetramer. SAMHD1 tetramer possesses four allosteric sites, where a total of 8 dGTP (two molecules per site) molecules bind. These interactions allow SAMHD1 tetramer to be stable for a long period of time, continuously hydrolyzing dNTPs (Yan et al., 2013; Hansen et al., 2014; Arnold et al., 2015). Structural comparison of the dimer and tetramer

elucidated how dGTP-binding at the two allosteric sites allows the catalytic site to be reconfigured for dNTP hydrolysis (Ji et al., 2013; Zhu et al., 2013).

Careful examination of the tetramer structure revealed distinctive hydrogen bonding networks between the Guanine base of dGTP and amino acid residues located at each of the two allosteric sites (Hansen et al., 2014). Three residues at the primary allosteric site, Asp137, Gln142, and Arg145, provide five hydrogen bonds, in total, to the Guanine base, which is the only base type found to fit this site (Figure 2A). The number and pattern of hydrogen bonds provided by amino acid residues at the secondary allosteric site, on the other hand, are flexible, and the site is able to accommodate all base types. Two triphosphates at both primary and secondary allosteric sites are coordinated by a single Mg^{2+} ion and form multiple hydrogen bonds with nearby basic residues. Notably, the guanine base binding specificity and other electrostatic static interactions with the triphosphate of the nucleotide at the primary site allows GTP, as well as dGTP, to bind with micromolar-affinity (Ji et al., 2014; Koharudin et al., 2014; Arnold et al., 2015; Zhu et al., 2015). SAMHD1 with GTP occupying the primary site is not only a catalytically more active enzyme but is also a more stable tetramer compared to SAMHD1 with dGTP at the primary site (Amie et al., 2013; Koharudin et al., 2014; Miazzi et al., 2014; Arnold et al., 2015). Since the cellular concentration of GTP is higher than that of dGTP, by a factor of several fold, GTP is likely the primary allosteric activator of SAMHD1 dNTPase in cells (Amie et al., 2013). However, the secondary allosteric site is strictly configured for the deoxyribose ring (Ji et al., 2014; Koharudin et al., 2014; Arnold et al., 2015; Zhu et al., 2015). Together, the crystal structure and biochemical studies suggest that GTP-binding at the primary site induces dimerization, which subsequently is followed by conversion to a catalytically active tetramer upon dNTP binding at the secondary allosteric site (Hansen et al., 2014; Zhu et al., 2015).

Unlike the allosteric sites, dNTP binding at the catalytic site is primarily mediated through the triphosphate of each dNTP (Figure 2B). A series of SAMHD1 crystal structures, with all possible dNTPs in the catalytic site, clearly illustrates the promiscuous substrate specificity (Ji et al., 2014; Wu et al., 2015). In these structures, a metal ion is coordinated by two histidine residues (H167, H206) and two aspartate residues (D207, D311) (Zhu et al., 2013).

In addition to its well-characterized dNTPase activity, the catalytic core domain has been reported to bind single-stranded DNA/RNA and to have exonuclease activity. While evidence for DNA/RNA binding is consistent, the assessment of its exonuclease activity has been controversial. Beloglazova et al., demonstrated that SAMHD1 possesses strong 3'-5' single-stranded DNA exonuclease activity and weaker 3'-5' single-stranded RNA exonuclease activity (Beloglazova et al., 2013). However, Ryoo and colleagues showed that SAMHD1 contains only RNA nuclease activity (Ryoo et al., 2014). In contrast to these reports, Seamon et al., reported that SAMHD1 has single-stranded nucleic acid binding activity without intrinsic exonuclease activity (Seamon et al., 2015). Despite the controversy over the protein's exonuclease activity, the quaternary state of the SAMHD1-nucleic acid complex has been consistently proposed to be different from the GTP/dNTP-induced tetramer. In particular, unlike their inducing effects on dNTPase activity and tetramerization, GTP and dGTP have been found to inhibit either single-stranded DNA and RNA nuclease

activity (Beloglazova et al., 2013; Ryoo et al., 2014) or single-stranded RNA binding activity (Seamon et al., 2015). Thus, SAMHD1 likely binds nucleic acids with a different quaternary structure (Seamon et al., 2015).

The C-terminus of SAMHD1

The C-terminus of SAMHD1 (residues 600–626), despite being included in the crystal structure of GTP/dNTP-bound tetramer, was not resolved. This region of the protein has evolved, along with the N-terminus, through positive selection with viral antagonist (Laguette et al., 2012; Lim et al., 2012). The crystal structure of a ternary complex of human SAMHD1 (residues 582–626), including the C-terminus, with Vpx of SIV from the sooty mangabey and human DCAF1 revealed that the C-terminus forms a short alpha-helical structure with an extended loop (Figure 1D) (Schwefel et al., 2014). While being divergent in sequence among vertebrate species, the C-terminus contains a conserved di-hydrophobic amino acid motif (Leu620-Phe621), recognized by cell cycle regulatory proteins (Yan et al., 2015). The biological significance of this motif and interaction will be discussed in the next section.

Mechanisms of HIV-1 restriction

SAMHD1 is generally accepted to restrict HIV-1 infection by controlling dNTP pools in immune cells, by cleaving dNTPs into deoxyribonucleosides and triphosphates (Goldstone et al., 2011; Lahouassa et al., 2012). Evidence for this mechanism of restriction comes from several sources. Laguette et al., demonstrated that the catalytic core domain is essential for HIV-1 restriction by showing that mutations of the histidine and aspartic acid residues (H206 and D207) cause the protein to lose its ability to restrict HIV-1 in U937 cells (Laguette et al., 2011). The crystal structures of the core domain illustrate that these two residues coordinate a metal ion at the catalytic site, providing a structural basis for this observation (Zhu et al., 2013) (Figure 2B). Additional support for this mechanism comes from SAMHD1 depletion studies. Knock-down of SAMHD1, by siRNA or by infecting cells with Vpx-containing virions, not only supports HIV-1 replication but also results in higher levels of dNTP pools (Lahouassa et al., 2012). Together, these correlative findings suggest that the molecular mechanism of HIV-1 restriction by SAMHD1 involves the protein's dNTPase activity, residing at the catalytic core domain.

The antiviral activity of SAMHD1 can be post-translationally modulated, primarily via phosphorylation at Thr592 by cyclinA2-CDK complex in cycling cells. Cyclin A2 binds the di-hydrophobic residues (Leu620-Phe621) at the C-terminus of SAMHD1 and CDK mediates phosphorylation at Thr592. A phosphomimetic substitution of Thr592 abrogates SAMHD1-dependent HIV-1 restriction, while a phosphorylation-defective mutant is able to restrict HIV-1 replication (Cribier et al., 2013; White et al., 2013). Furthermore, active phosphorylation of Thr592 has been observed in permissive cells (Cribier et al., 2013). However, the mechanism by which phosphorylation down-modulates HIV-1 restriction function is not clear. It was first suggested that phosphorylation does not alter the dNTPase activity of SAMHD1, *in vitro* as well as *in vivo* (White et al., 2013). On the other hand, structural and biochemical studies argued that the phosphomimetic mutations reduce

dNTPase activity by destabilizing the SAMHD1 tetramer, the catalytically active form of the protein (Arnold et al., 2015; Tang et al., 2015; Yan et al., 2015). Significantly, SAMHD1 tetramer with phosphorylation at Thr592 dissociates into catalytically inactive monomer and dimer more rapidly than wild-type (Arnold et al., 2015). The structural basis of this reduced tetramer stability is elegantly illustrated by comparing the crystal structures of tetramer with and without the phosphorylation. The modification not only causes a small rotation of one dimer relative to the other dimer in the tetramer, but also induces disordering and loss of tetramer stabilizing interactions of the C-terminus (Arnold et al., 2015). Consistent with structural data, their biochemical analysis showed that phosphorylated SAMHD1 tetramer is more sensitive to fluctuations in dNTP levels compared to wild-type. Thus, while both forms of SAMHD1 tetramer, phosphorylated or non-phosphorylated, are catalytically competent to a similar degree, upon depletion of dNTP pools, phosphorylated SAMHD1 tetramer will rapidly dissociate to an inactive form, defective in HIV-1 restriction (Arnold et al., 2015). On the other hand, in non-permissive and non-cycling cells, where the levels of Cyclin A2/CDK are low, unmodified SAMHD1 forms a long-lived, stable and catalytically active tetramer (Hansen et al., 2014; Arnold et al., 2015).

A second mechanism for SAMHD1-mediated HIV-1 restriction, one that does not involve the protein's dNTPase activity, was proposed in 2014, by Ryoo and coworkers, who found that SAMHD1 possesses RNA binding capability and suggested that it also has RNase activity (Ryoo et al., 2014). Specifically, Ryoo and colleagues engineered SAMHD1 proteins specifically lacking either the dNTPase activity or its proposed RNase activity and demonstrated that the mutant lacking dNTPase activity, but not the one lacking RNase activity, was still functional in HIV-1 restriction. They also showed that phosphorylation at Thr592 negatively modulates the RNase activity. The authors concluded that SAMHD1 possesses RNase activity and that the activity is essential for HIV-1 restriction, whereas the dNTPase activity is not required. However, these results were disputed by Seamon et al., who confirmed single-stranded DNA/RNA binding by SAMHD1 but argued that nuclease activity is not associated with the protein (Seamon et al., 2015). Their argument against endogenous SAMHD1 nuclease activity was based on a comprehensive biochemical analysis. In particular, SAMHD1 wild-type, catalytic site mutants (H206A/D207A, D207A), and a reported RNase/DNase defective mutant (Q548A) were purified over three orthogonal column chromatography steps. At each step, three enzymatic activities, dNTPase and single-stranded RNA and DNA nuclease activity, were assayed. While the dNTPase and RNase activity of wild-type SAMHD1 persisted over all chromatography steps, the DNase activity gradually diminished. Strikingly, catalytic site mutants and the reported RNase defective mutant displayed RNase activity similar to wild-type throughout purification. Their results suggest that SAMHD1 is not a nuclease, and even if it is, the putative RNase activity sites are not utilized for this activity (Seamon et al., 2015). Despite the conflicting data on nuclease activity, SAMHD1 does appear to bind single-stranded RNA. The molecular determinants of this binding and any associated HIV-1 restriction mechanism remain to be elucidated.

dNTP pools are controlled by ribonucleotide reductase (RNR) and SAMHD1

The levels of dNTPs, building blocks of DNA, are tightly regulated by the enzyme, RNR, discovered 54 years ago. RNR catalyzes conversion of all four ribonucleoside diphosphates into deoxyribonucleoside diphosphates, which are subsequently converted to dNTPs by nucleoside diphosphate kinase. The activity and cellular level of RNR is dynamically regulated, maximizing at the S-phase, when cells replicate nuclear DNA (Larsson et al., 2004). As cells transition from the S-phase to a quiescent state, the cellular levels of SAMHD1 increase, an opposite trend of the RNR (Franzolin et al., 2013). A reduction in SAMHD1, by siRNA in quiescent cells, results in a large increase in dNTP pools. It has long been thought that control of dNTP pools in cells is single-handedly controlled by RNR. However, the discovery of SAMHD1, a dNTPase, added another dimension to control of dNTP pools during the cell cycle.

Future prospects

Since SAMHD1 was discovered as a potent HIV-1 restriction factor in 2011, a great deal of knowledge about its structure, and regulation of its function, has accumulated in a relatively short time. However, the precise molecular mechanism of SAMHD1-mediated HIV-1 restriction is not clearly defined. SAMHD1 is likely to inhibit HIV-1 replication at multiple level with its dNTPase, single-stranded DNA/RNA binding, and nuclease activities. Structural information of nucleic acid-bound SAMHD1 will shed light on how these activities are inter-related.

While the N-terminus of SAMHD1 has undergone positive selection pressure through an evolutionary arms race with viral proteins, this region has not been demonstrated to be essential for SAMHD1-mediated HIV-1 restriction. However, the C-terminus, which has been under the same evolutionary pressure, is critical in stabilizing SAMHD1 tetramer, the catalytically active form of the dNTPase. The C-terminus also harbors a docking site for the Cyclin A-CDK complex, a modulator of SAMHD1 HIV-1 restriction activity (Yan et al., 2013, 2015). Structures of full-length SAMHD1 in different quaternary states will address the functional significance of the N-terminus in SAMHD1 biology.

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Figure 1. SAMHD1 domain structures

(A) A schematic diagram of SAMHD1 comprising three domains; the N-terminus (purple), catalytic core (blue), and the C-terminus (yellow). The amino acid residue numbers at the borders of three domains are based on the crystal structures of the catalytic core domain of SAMHD1. Several residues that have been characterized for their roles in SAMHD1 function are indicated. The nuclear localization signal (NLS) comprises residues 11–14. Residues D137, Q142, and R145, are located at the primary GTP/dGTP-binding allosteric site, and residue N119, R333, and N358 are at the secondary dNTP-binding allosteric site. The residues at the catalytic site are Q149, R164, H206, D207, H210, H215, H233 and Q375. Residue Q548 has been implicated in the putative nuclease activity. In cycling cells, T592 is phosphorylated by cyclin A2/CDK which binds at L620-F621. The C-terminal residues R617, L620, and F621 are recognized by Vpx for proteasome-dependent down-regulation. (B) The crystal structure of the mandrill monkey SAMHD1 N-terminus (PDB code 5AJA). The structure was solved in complex with the Vpx of an SIV strain that infects mandrill monkey and with the C-terminus of human DCAF1. The structure of SAMHD1 residues P34-Q109 is shown. Residues S23-S33 were not resolved in the structure. (C) The crystal structure of tetrameric human SAMHD1 catalytic core in complex with dGTP (PDB code 4BZB). Each monomer is identified with distinct colors. Three monomers form an allosteric site comprising a primary and secondary allosteric site, occupied by two dGTP molecules (sphere). The catalytic site in each monomer is occupied by dGTP. (D) The crystal structure of the C-terminus of human SAMHD1 (PDB code 4CC9). The structure was solved in complex with Vpx of an SIV strain that infects sooty mangabey monkey and the C-terminus of human DCAF1.

Figure 2.

Allosteric site structure (A) and catalytic site structure (B) of tetrameric SAMHD1 in complex with GTP and dATP. (A) Hydrogen bond interactions (lines in yellow) in the allosteric site with GTP at the primary site and dATP at the secondary site (PDB code 4QG1). A Mg^{2+} ion (sphere in green) is coordinated with the two triphosphates of GTP and dATP. (B) Hydrogen bond interactions in the catalytic site with dGTP (PDB code 4MZ7). A metal ion (blue sphere) is coordinated with histidine and aspartic acid residues (H167, H206, D207, and D311).