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Impaired dNTPase Activity of SAMHD1 by Phosphomimetic Mutation of Thr-592*◆

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Background: Phosphorylation of SAMHD1 Thr-592 inhibits its anti-HIV activity.
Results: Phosphomimetic mutation T592E of SAMHD1 perturbs SAMHD1 crystal structure, destabilizes SAMHD1 tetramer, and reduces its dNTP triphosphatase (dNTPase) activity.
Conclusion: T592E decreases the dNTPase activity of SAMHD1 via destabilizing the catalytically active tetramer.
Significance: Structure-induced impairment of SAMHD1 dNTPase activity by T592E suggests a mechanism of the phosphorylation-regulated SAMHD1 antiviral activity.

SAMHD1 is a cellular protein that plays key roles in HIV-1 restriction and regulation of cellular dNTP levels. Mutations in SAMHD1 are also implicated in the pathogenesis of chronic lymphocytic leukemia and Aicardi-Goutières syndrome. The anti-HIV-1 activity of SAMHD1 is negatively modulated by phosphorylation at residue Thr-592. The mechanism underlying the effect of phosphorylation on anti-HIV-1 activity remains unclear. SAMHD1 forms tetramers that possess deoxyribonucleotide triphosphate triphosphohydrolase (dNTPase) activity, which is allosterically controlled by the combined action of GTP and all four dNTPs. Here we demonstrate that the phosphomimetic mutation T592E reduces the stability of the SAMHD1 tetramer and the dNTPase activity of the enzyme. To better understand the underlying mechanisms, we determined the crystal structures of SAMHD1 variants T592E and T592V. Although the neutral substitution T592V does not perturb the structure, the charged T592E induces large conformational changes, likely triggered by electrostatic repulsion from a distinct negatively charged environment surrounding Thr-592. The phosphomimetic mutation results in a significant decrease in the population of active SAMHD1 tetramers, and hence the dNTPase activity is substantially decreased. These results provide a mechanistic understanding of how SAMHD1 phosphorylation at residue Thr-592 may modulate its cellular and antiviral functions.

SAMHD1 is a retroviral restriction factor that is ubiquitously expressed in most human cell types (1–6). It blocks infection by HIV-1 and certain DNA viruses, such as herpes simplex virus type 1, in non-dividing myeloid-lineage cells and/or quiescent $CD4^+$ T-lymphocytes (7, 8). It also plays other important roles in the human innate immune system (9, 10) and is implicated in maintaining genome stability (11, 12). Mutations in SAMHD1 are associated with the autoinflammatory condition Aicardi-Goutières syndrome (13) and a progression of chronic lymphocytic leukemia (14). The deoxyribonucleotide triphosphate triphosphohydrolase (dNTPase) activity of SAMHD1 degrades dNTP into deoxynucleoside and inorganic triphosphate (15, 16), which tightly regulates the dNTP levels in non-dividing cells (11). SAMHD1 has also been reported to bind and degrade ssDNA (17) and ssRNA (17, 18), although its nuclease activity is currently under dispute (15, 16, 19, 20).

SAMHD1 contains an N-terminal sterile alpha motif (SAM)[2] and a C-terminal histidine-aspartic (HD) domain that is sufficient for dNTPase activity and HIV-1 restriction (21). The tetrameric form of SAMHD1 is catalytically active, and the dNTPase activity of SAMHD1 is regulated by the combined action of GTP and all four dNTPs (22–26). The structures of the nucleotide-bound catalytic core of the SAMHD1 tetramer revealed four copies of three unique ligand binding sites, including two allosteric sites for the binding of a pair of nucleotides and one catalytic site. The binding of the allosteric nucleotides at the junction of three subunits induces large conformational changes that result in the catalytically active SAMHD1 (25, 27).

Although it is well established that SAMHD1 does not restrict HIV-1 in dividing cells where the protein is phosphorylated at residue Thr-592 (21, 28–32), the effect of this phosphorylation on SAMHD1 dNTPase activity is unclear. The phosphomimetic mutants of SAMHD1 (T592E/T592D) impair the ability to block HIV-1 infection in non-dividing cells, but were observed to retain the ability to hydrolyze dNTPs *in vitro* and reduce the cellular dNTP levels (31, 33). In contrast, disruption of SAMHD1 Thr-592 phosphorylation either by a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor or by SAMHD1 mutants defective for cyclin A2 interaction results in

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The atomic coordinates and structure factors (codes 4ZWG and 4ZWE) have been deposited in the Protein Data Bank (http://wwpdb.org/).
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[2] The abbreviations used are: SAM, sterile alpha motif; HD, histidine-aspartic; CDK, cyclin-dependent kinase.

a substantial reduction of the intracellular level of dNTPs (30, 34), supporting the hypothesis that phosphorylation impairs the dNTPase activity of SAMHD1. The cell cycle-dependent intracellular dNTP levels and activation of CDKs also hint at a potential link between SAMHD1 phosphorylation and its dNTPase activity. The activities of CDKs control cell cycle progression and phosphorylation of SAMHD1 (11). These events synchronize with the changes in the cellular dNTP levels (11, 35), presumably affected by phosphorylation-regulated modulation of the dNTPase activity of SAMHD1.

To investigate the effect of SAMHD1 Thr-592 phosphorylation on its dNTPase activity and the mechanism underlying phosphorylation-regulated HIV-1 restriction by SAMHD1, we performed structural and enzymatic studies on SAMHD1 mutants T592E and T592V. The results demonstrate that the folding of the SAMHD1 region around T592E, but not T592V, is disrupted, likely due to negative charge repulsion generated by the phosphomimetic mutation. This disruption leads to substantial destabilization of the active tetrameric form of SAMHD1 and an ~3-fold decrease in its dNTPase activity. The reduction in dNTPase activity of the Thr-592 phosphomimetic mutant of SAMHD1 suggests a correlation between the dNTPase activity and the HIV-1 inhibition function by SAMHD1, shedding light on the phosphorylation-regulated mechanism of SAMHD1 in cell cycle control and HIV-1 restriction.

Experimental Procedures

Protein Expression and Purification—SAMHD1 (residues 1–626) and SAMHD1c (residues 113–626) constructs were cloned into a pET28a expression vector with a His_6 tag at the N terminus. Site-directed mutagenesis was performed to introduce the indicated mutations as described previously (25). All proteins were expressed in *Escherichia coli* and purified using a nickel-nitrilotriacetic acid affinity column. The eluted peak fractions were collected and dialyzed into a buffer containing 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM $MgCl_2$, and 0.5 mM tris-(2-carboxyethyl)phosphine (assay buffer) and then further purified with size-exclusion chromatography as described previously (26).

Crystallization and Data Collection—SAMHD1c-RN (residues 113–626 with mutations H206R/D207N) with T592E/T592V mutation was mixed with either 4 mM dGTP or a mixture of 4 mM GTP and 4 mM dATP (final concentrations) in the assay buffer. Crystals were grown at 25 °C using the microbatch under-oil method by mixing 1 μl of protein (3 mg/ml) with 1 μl of crystallization buffer (100 mM SPG (Qiagen) buffer, pH 7.4, 25% PEG 1500). Crystals were cryo-protected by adding 25% (v/v) glycerol before frozen in liquid nitrogen. Diffraction data were collected at the Advanced Photon Source beamline 24-ID-C (wavelength 0.979 Å and temperature 100 K). The data statistics are summarized in Table 1.

Structure Determination and Refinement—The structures were solved by molecular replacement using Phaser (36). One subunit of the previously published SAMHD1 structure (PDB (Protein Data Bank) ID 4BZB) was used as the initial search model, with all bound nucleotides removed. The initial model was refined with iterative rounds of TLS (translation/libration/screw) and noncrystallographic symmetry-restrained refinement using Refmac5 (37), together with manual rebuilding using Coot (38). Refinement statistics are summarized in Table 1.

Analytical Size-exclusion Chromatography—Purified samples of SAMHD1-RN with or without the T592E/T592V mutation (2 mg/ml, 200 μl) were pre-incubated with GTP and dATP at a final concentration of 100 μM each and then injected onto an analytical Superdex 200 10/300 GL column (GE Healthcare), which is pre-equilibrated in assay buffer. The elution profiles (UV absorbance at 280 nm) were recorded.

Analytical Ultracentrifugation—Sedimentation velocity experiments were performed using a Beckman XL-I analytical ultracentrifuge with an An60-Ti rotor at 20 °C. All data were collected at 42,000 rpm and analyzed using the program SEDFIT (39). Protein samples were concentrated to 1 mg/ml in assay buffer containing GTP and dATP, each at a final concentration of 100 μM. SEDNTERP was used to calculate the experimental parameters including sample partial specific volume, buffer density, and viscosity.

SAMHD1 dNTPase Activity Assays—Standard SAMHD1 dNTPase activity assay conditions contained 0.5 mM GTP, 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM $MgCl_2$, and 0.5 mM tris-(2-carboxyethyl)phosphine in a 500-μl reaction volume, with substrate dNTP at indicated concentrations. Reactions were initiated at 37 °C by the addition of purified SAMHD1 samples to a final concentration of 500 nM. Aliquots of reactions were removed at various time points and terminated by 5× dilution into ice cold buffer containing 10 mM EDTA, followed by deproteinization by spinning through an Amicon Ultra 0.5-ml 10-kDa filter (Millipore) at 16,000 × *g* for 20 min. Deproteinized samples were analyzed by HPLC using a Synergi C18 column 150 × 4.6 mm (Phenomenex), pre-equilibrated in 20 mM ammonium acetate, pH 4.5 (buffer A). Samples were eluted with a methanol (buffer B) gradient over 14 min at a flow rate of 1 ml/min. The elution profiles (UV absorption at 260 nm) were recorded as described previously (40).

Results

The Phosphomimetic T592E Mutation Impairs SAMHD1 dNTPase Activity in Vitro—Understanding the direct effect of Thr-592 phosphorylation on SAMHD1 dNTPase activity is an important step in understanding the exact mechanism of viral restriction. Therefore, we generated the phosphomimetic mutant T592E and the neutral mutant T592V of SAMHD1 and compared their dNTPase activities with that of WT enzyme. Recombinant SAMHD1 variants of high purity (Fig. 1*A*) were incubated with 500 μM GTP and 1 mM dNTP, and the reaction product deoxynucleoside was subsequently monitored (26). The T592E mutant was still able to hydrolyze dGTP, but the hydrolysis rate decreased substantially when compared with WT SAMHD1 and T592V mutant (Fig. 1*B*). A similar decrease in dNTPase activity has been reported for the phosphomimetic mutant T592D (34). As T592E has a higher structural similarity to phosphothreonine due to its longer side chain than that of T592D, we therefore focused on the structural studies of T592E. The decrease in dNTPase activity of T592E mutant was observed for all of the four dNTPs (Fig. 1*C*). We further incu-

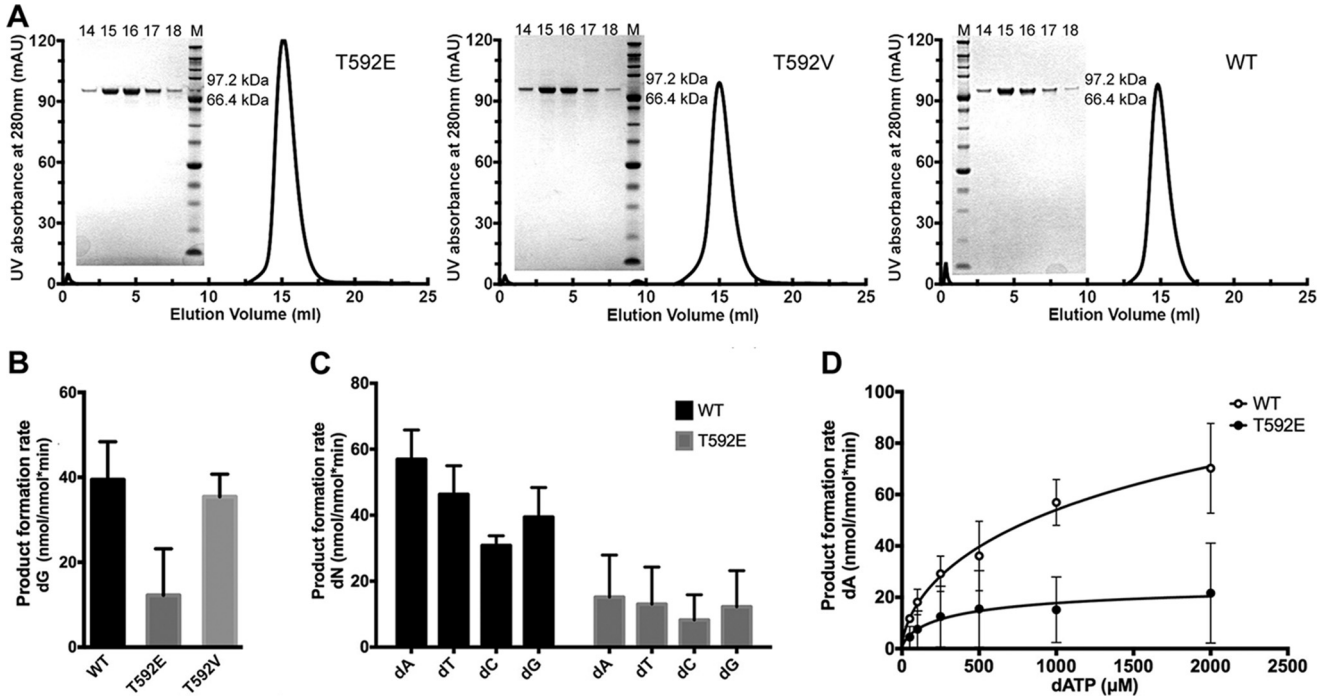

FIGURE 1. **Phosphomimetic mutation T592E impairs SAMHD1 dNTPase activity.** *A*, the purity of recombinant SAMHD1 variants T592E (*left*), T592V (*middle*), and WT (*right*) enzyme as demonstrated by analytical size-exclusion chromatography (*left*) and SDS-PAGE (*right*). SAMHD1 variants (200 μl) at 2 mg/ml were applied to a Superdex 200 10/300 GL column. Fraction numbers on the SDS-PAGE indicate elution volume. *Lane M* indicates molecular weight markers. *B*, the dNTPase activity of WT SAMHD1 or the T592E/T592V mutant (0.5 μM) was assayed with 1 mM dGTP for 5–15 min. *C*, the dNTPase activity of WT SAMHD1 or the T592E mutant (0.5 μM) was assayed with 1 mM dNTP and 500 μM GTP for 5–15 min. *dA*, deoxyadenosine; *dT*, deoxythymidine; *dC*, deoxycytidine; *dG*, deoxyguanosine. *D*, the dNTPase activity assays were performed in the presence of 500 μM GTP and the indicated concentrations of dATP. In each experiment, the amount of deoxynucleoside products generated in the reactions was quantified by HPLC. Each experiment was repeated with three independent batches of recombinant enzymes. *Error bars* represent S.E. from triplicate experiments.

bated WT or T592E SAMHD1 with 500 μM GTP and dATP at various concentrations (0–2000 μM) and measured the steady state kinetic rates of the reaction (Fig. 1*D*). When compared with the deoxyadenosine production rates of WT SAMHD1, the phosphomimetic mutant showed a decrease by about 3-fold at all tested concentrations. As WT and T592V SAMHD1 restrict HIV-1, whereas T592E impairs HIV-1 restriction (31, 33), our activity results indicate a correlation between phosphorylation-regulated SAMHD1 dNTPase activity and the ability to restrict HIV-1 infection.

The Phosphomimetic Mutation T592E Causes Large Structural Perturbations at the SAMHD1 Tetramer Interface—To investigate the potential structural effect of Thr-592 phosphorylation on SAMHD1, we created the T592E and T592V variants on the background of the SAMHD1 catalytic core construct SAMHD1c-RN. SAMHD1c-RN has been shown previously to be identical in conformation and nucleotide binding properties to the WT enzyme, but it is more amenable to crystallization (25, 26). We determined the crystal structures of the mutants T592E (with GTP and dATP) and T592V (with dGTP) at 2.3 and 2.8 Å resolution, respectively (Table 1). Similar to SAMHD1c-RN in complex with the same nucleotides (PDB ID: 4TNR and 4BZB), both T592E and T592V crystallized as tetramers in the space group $P2_1$ with closely related cell dimensions (Fig. 2). The structure of the T592V mutant is virtually identical to that of WT SAMHD1c-RN (Fig. 2*B*) and thus will not be described in detail. However, in contrast to the stable structure of SAMHD1c-RN, the regions around the phosphomimetic sites were largely disordered in the T592E structure (Fig. 2*A*). Nonetheless, the allosteric and substrate nucleotides bound to the T592E mutant were clearly

TABLE 1
Crystallographic data collection and refinement statistics
dA, deoxyadenosine; dG, deoxyguanosine.

Data	T592E-dA[a]	T592V-dG[a]
Data collection		
Space group	$P2_1$	$P2_1$
Cell	79.7, 137.0, 95.8	89.1, 146.1, 98.5
a, *b*, *c* (Å)	90.0, 114.0, 90.0	90.0, 115.0, 90.0
α, β, γ (°)		
Resolution (Å)	50.0-2.30 (2.34-2.30)	50.0-2.81 (2.85-2.80)
Unique reflections	82,062	54,764
R_{merge}	0.174 (–[b])	0.194 (–[b])
$I/\sigma I$	8.4 (1.3)	8.7 (1.2)
Completeness (%)	98.4 (97.6)	98.7 (99.7)
Redundancy	3.4 (3.2)	6.9 (7.1)
Refinement		
No. of reflections	77,952	51,938
R_{work}/R_{free}	0.20/0.24	0.21/0.25
No. of atoms		
Protein	15104	15718
Ligand	372	376
Water	280	14
B-factors		
Protein	33	70
Ligand	32	69
Water	35	42
RMSD[c]		
Bond lengths (Å)	0.011	0.010
Bond angles (°)	1.6	1.5
PDB ID code	4ZWG	4ZWE

[a] Numbers in brackets are for the highest resolution shell.
[b] $R \geq 1$.
[c] RMSD, root mean square deviation.

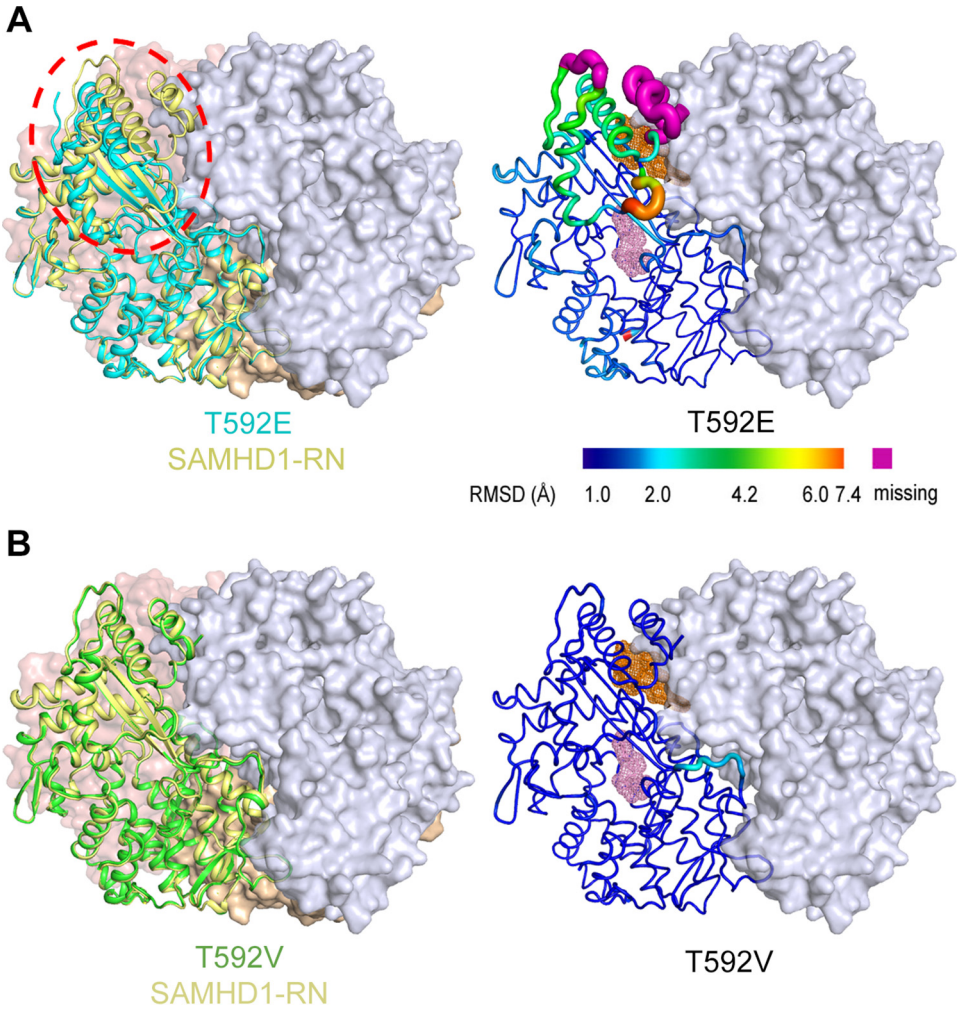

FIGURE 2. **Crystal structures of the SAMHD1 T592E and T592V tetramers.** *A*, *left*, structural superposition of SAMHD1 T592E (*cyan*) and SAMHD1c-RN (residues 113–626 with mutations H206R/D207N) (*yellow*). The tetramer structure is shown as ribbon for one subunit and surface for the other three subunits. The large conformational change is marked by a *red oval*. *Right*: putty and surface representations of the overlay of T592E and SAMHD1c-RN subunits. T592E is structurally aligned to SAMHD1c-RN using SHP (45). The color spectrum and the coil thickness represent the root mean square deviation (*RMSD*) of the aligned Cα atoms. The disordered regions in the T592E structure are shown in *magenta*. The bound nucleotides near the subunit interface are shown as meshes. *B*, the comparison of SAMHD1 T592V (*green*) and SAMHD1c-RN (*yellow*) shows virtually no structural changes.

observed in unperturbed conformations, as were those in the SAMHD1c-RN structure, with a root mean square deviation of 0.7 Å (Fig. 3, *A* and *B*).

The phosphomimetic mutation T592E leads to disordered regions and structural perturbations at the tetramer interface of SAMHD1 (Fig. 2*A*). We refer to the tetramer interface as the region formed between two inactive SAMHD1 dimers (25). The residues beyond Pro-581 in the T592E structures, including the phosphorylation site Thr-592, are disordered and unstructured. In contrast, residues 582–599 were clearly observed at the tetramer interface in the SAMHD1c-RN structure (25). The residues 583–599 are also missing in the inactive SAMHD1 dimer structure (15) and become structured upon tetramerization of SAMHD1 (25). Importantly, this conformational change leads to additional structural perturbation, with a neighboring region including three α helices and two β strands (residues 453–506 and 546–581) shifted away from the tetramer interface by about 3 Å. The perturbation results in a large reduction of ~20% (1136 Å^2) in buried surface area at the tetramer interface, potentially destabilizing the SAMHD1 tetramer.

The conformational changes at the tetramer interface of SAMHD1 T592E lead to little structural perturbation to the conformation of the bound nucleotides, but make the allosteric nucleotide more solvent-accessible (Fig. 3*C*). The bound nucleotides and the surrounding residues at the allosteric sites and the catalytic sites overlay well between the T592E and SAMHD1c-RN structures (Fig. 3, *A* and *B*). However, the conformational changes in the T592E structure open up the otherwise mostly buried allosteric sites, making them partially solvent-accessible and presumably destabilizing the bound allosteric nucleotides (Fig. 3*C*). Both the reduction of the tetramer interface and the destabilization of allosteric nucleotides will lead to a decreased population of the catalytically active SAMHD1 tetramer. These observations suggest that phosphorylation at Thr-592 does not directly affect the catalysis but rather the stability of active SAMHD1 tetramer.

The Phosphomimetic Mutation T592E Reduces the Stability of SAMHD1 Tetramer in Solution—As our structural data suggest that the T592E tetramer is unstable, we investigated the formation and stability of the T592E phosphomimetic

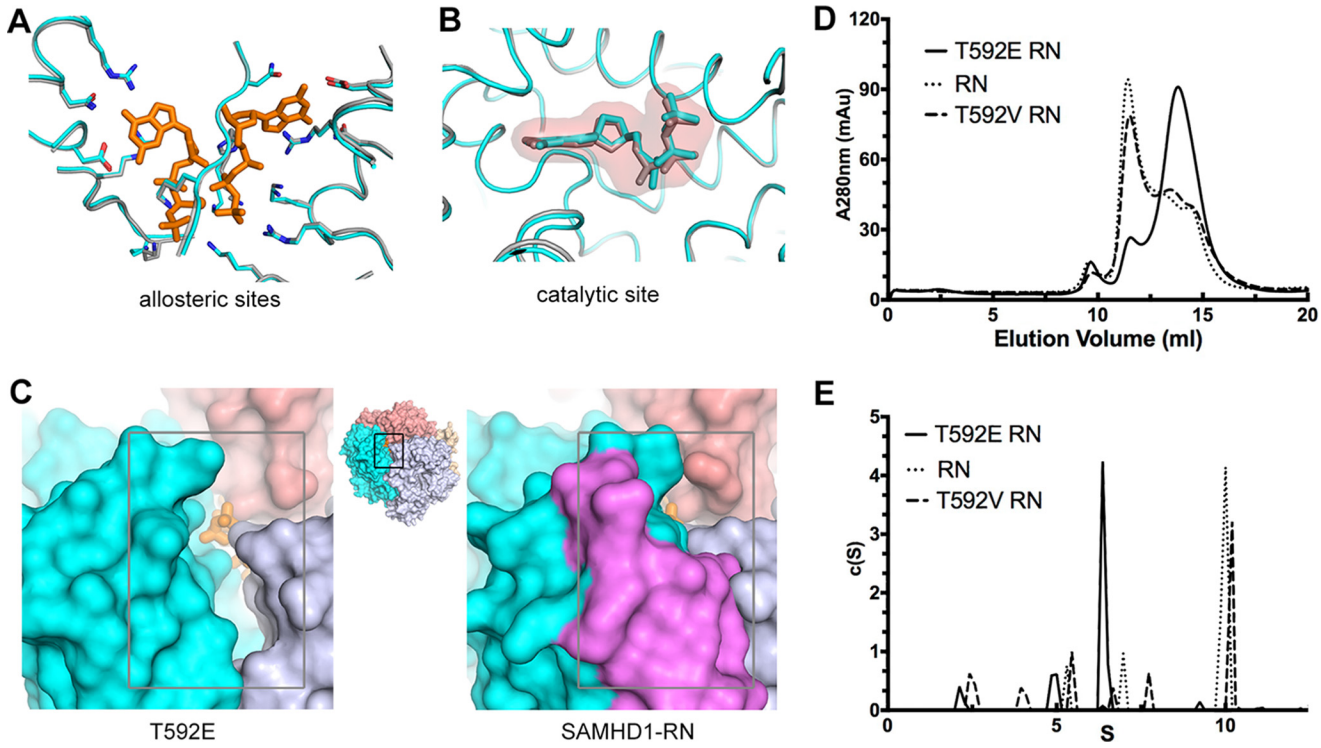

FIGURE 3. **Phosphomimetic T592E mutation impairs SAMHD1 tetramer stability.** *A*, superposition of allosteric sites in the T592E (*cyan*) and SAMHD1c-RN (*gray*) structures. Proteins are shown as ribbon with selected side chains as sticks. *B*, overlay of the substrate binding pockets in the two structures. *C*, partial exposure of the allosteric sites in SAMHD1 T592E. The allosteric site of T592E (*left*) and SAMHD1c-RN (*right*), and the overview of the T592E tetramer (*middle*), are shown with subunits colored individually and allosteric nucleotides as *orange sticks*. The SAMHD1c-RN region missing in the T592E structure is highlighted in *magenta*. *D*, size-exclusion chromatograms of SAMHD1-RN and its T592E/T592V variants in the presence of GTP and dATP. Purified samples of SAMHD1 (2 mg/ml, 200 μl) mixed with a final concentration of 4 mM GTP and 4 mM dATP were applied to a Superdex 200 10/300 GL column. *mAu*, milliabsorbance unit. *E*, sedimentation velocity results for SAMHD1-RN and its T592E/T592V variants (1 mg/ml) in the presence of GTP (100 μM) and dATP (100 μM).

SAMHD1 tetramer *in vitro*. We generated the Thr-592 variants on the background of the full-length SAMHD1-RN and compared their oligomerization states with that of SAMHD1-RN using both size-exclusion chromatography and sedimentation velocity analytical ultracentrifugation. Even upon incubation with the nucleotides that induce tetramer formation, the majority of the T592E protein remained dimeric, with the amount of tetramer reduced substantially (Fig. 3, *D* and *E*). In contrast, under the same conditions, the majority of the T592V mutant and SAMHD1-RN formed a tetramer (Fig. 3, *D* and *E*). Consistent with our structural data, these results demonstrated that, although the phosphomimetic mutant of SAMHD1 can form an active tetramer under our experimental conditions, the stability of the tetramer is substantially reduced.

Electrostatic Repulsion by Negatively Charged Modification at Thr-592 Destabilizes SAMHD1 Tetramer—The tetramer-destabilizing effect of Thr-592 phosphorylation is potentially due to the disruption of the folding of an important SAMHD1 C-terminal region (residues 559–599) at the tetramer interface (Fig. 4). This helix-turn-helix region contains three short α-helices (Fig. 4*A*). Thr-592 is located at the center of this region and is surrounded by a large number of main-chain carbonyl groups that point in the same direction as the surface-exposed Thr-592 side chain (Fig. 4*B*). As each main-chain carbonyl group has a partial negative charge (41), this creates a striking and definite negatively charged patch on the protein surface around Thr-592 (Fig. 4*C*), which is very rigid in geometry. The acidic side chain of neighboring Asp-585 also points in the same direction, although it is likely neutralized by the nearby Lys-580. The introduction of additional negative charge at Thr-592, by either phosphorylation or the phosphomimetic glutamate substitution, results in strong electrostatic repulsions to the surrounding main-chain arrangement that disrupt the folding of this tetramer interface region. Consistent with this notion, the T592V structure shows that the neutral substitution at this site does not disturb the structure, leaving the tetramer interface intact (Fig. 4*A*). This agrees with the observations that neutral Thr-592 substitutions (Val and Ala) have little or no effect on SAMHD1-mediated HIV-1 restriction (31–33). Therefore, the distinct negatively charged environment surrounding Thr-592 provides a mechanistic explanation to the destabilizing effect of phosphorylation at this site.

Discussion

SAMHD1 has been identified as a retroviral restriction factor (2–6) and a key regulator of the cellular dNTP pools (11). The dNTPase activity of SAMHD1 is triggered by a nucleotide-induced tetramerization through the combined action of GTP and all four dNTPs (24, 26). Residue Thr-592 of SAMHD1 is phosphorylated by CDKs in cycling cells, in which SAMHD1 does not restrict HIV-1 infection. How SAMHD1 phosphorylation regulates its activities is unclear. Although some studies suggested that phosphorylation compromised the ability of SAMHD1 to restrict retroviruses without altering its dNTPase

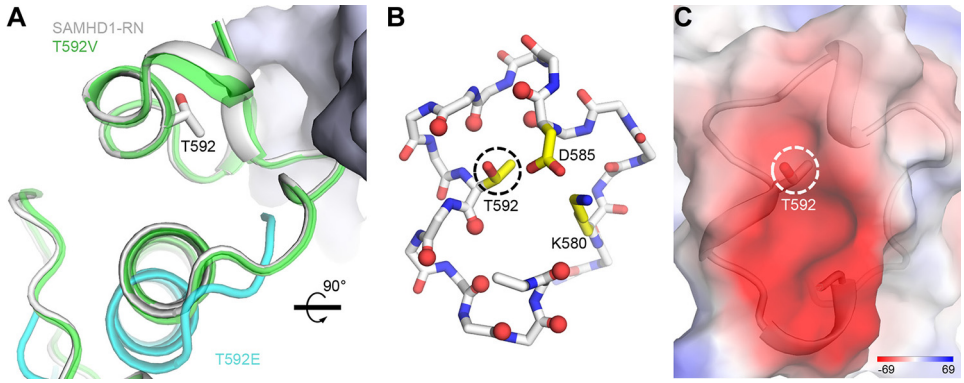

FIGURE 4. **Structural environment of SAMHD1 residue Thr-592.** *A*, the helix-turn-helix region at SAMHD1 C terminus is shown as *ribbon* with the neighboring subunit as surface. The structure of SAMHD1c-RN (*white*) is superposed to those of T592E (*cyan*) and T592V (*green*). Thr-592 is shown in sticks. *B*, stick representation of the same region in an orthogonal view. Only the surface-exposed main chains are displayed to simplify the view, except for Thr-592, Asp-585, and Lys-580 with side chain shown in *yellow*. The main-chain carbonyl groups contributing to the negatively charged patch around Thr-592 are highlighted as *red spheres*. *C*, surface and ribbon representations of the region shown in the same orientation as in *panel B*; the surface is colored according to electrostatic surface potentials (unit K_bT/e_c). Thr-592 is marked by a *black* or *white circle* (*B* and *C*).

activity (31, 33), others posit that the loss of retroviral restriction is linked to decreased dNTPase activity upon SAMHD1 phosphorylation (29, 30, 34). These discrepancies might be due to differences in assay conditions (such as cell differentiation into non-dividing status) and interpretation of results, or the contribution of additional cellular factors that are involved *in vivo*. There are few structural or *in vitro* biochemical data that rigorously explain the phosphorylation effect on SAMHD1 function.

The work presented herein provides detailed structural and biochemical evidence that establishes a link between SAMHD1 phosphorylation and its dNTPase activity. Our results demonstrate that the phosphomimetic mutation T592E destabilizes the active SAMHD1 tetramer and substantially decreases its dNTPase activity. Importantly, the structural analysis offers a mechanistic foundation for understanding the direct effect of Thr-592 phosphorylation. As Thr-592 is located in a highly negatively charged region near the SAMHD1 tetramer interface, the additional negative charge resulting from phosphorylation will generate repulsive forces that disrupt the folding of the region. This disruption destabilizes the tetramer required for the dNTPase activity. It is also possible that Thr-592 phosphorylation and the associated structural changes affect other cellular functions or interactions of SAMHD1. Further studies are needed to fully understand the role of this post-translational modification of SAMHD1.

Our results on T592E are consistent with the reported effect of SAMHD1 T592D, which reduces the dNTPase activity *in vitro* and maintains the ability to decrease the cellular dATP level, but to a lesser extent than SAMHD1 non-phosphorylatable variants (34). Thus, there is growing evidence suggesting that Thr-592 phosphomimetics reduce the population of the dNTPase-active SAMHD1 by destabilizing the SAMHD1 tetramer. Our study reveals the delicate nature of the Thr-592 location. We also show that different substitutions (T592E and T592V) affect the structure and enzymatic activity differently. Whether the phosphomimetic effect precisely reflects that of Thr-592 phosphorylation remains to be investigated. It is possible that other unknown cellular co-factors may contribute to the modulation of the phosphorylation-regulated SAMHD1 enzymatic activity in cells.

The modulation of the SAMHD1 dNTPase activity by phosphorylation can facilitate the progression of genome replication and genome integrity. Appropriate levels of cellular dNTPs, which vary drastically during the cell cycle, are of paramount importance to cell fate and fitness. Tightly controlling SAMHD1 activity is critical for the regulation of intracellular dNTP pools. Phosphorylation may have evolved as a method to quickly deactivate SAMHD1 when necessary during cell cycle. In cycling cells, CDK2 coordinates entry into S phase, whereas CDK1/CDK2 are essential for the completion of the S phase (42). Phosphorylation of SAMHD1 at Thr-592 by the CDK1 and/or CDK2 complex may enable cells to down-regulate its dNTPase activity during S phase, when high intracellular dNTP levels are required for efficient genome replication.

The SAMHD1 activity responsible for retroviral restriction in non-dividing cells is under debate, with studies indicating that either the dNTPase activity alone (5, 15, 16, 43, 44) or the RNase (18) activity is essential for HIV-1 restriction. A recent biochemical study suggests that recombinant SAMHD1 does not possess a nuclease activity (20). Nevertheless, it is clear that SAMHD1 does not show viral restriction ability in cycling cells where it is phosphorylated at Thr-592 by CDK1, CDK2, and CDK6 (28–33). Our findings indicate that Thr-592 phosphorylation likely affects the SAMHD1 dNTPase activity, which may partially account for the impaired retroviral restriction ability of SAMHD1 in cycling cells. More functional studies are required to fully unveil the precise mechanism underlying phosphorylation-modulated retroviral restriction of SAMHD1.

Author Contributions—C. T., X. J., L. W., and Y. X. designed the research; C. T. performed the research; C. T., X. J., L. W., and Y. X. analyzed the data; and C. T., X. J., L. W., and Y. X. wrote the paper.

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