SAMHD1's Interaction with Nucleotide Analogue Molecule X

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Abstract

SAMHD1 is an enzyme that regulates deoxyribonucleotide triphosphate (dNTP) concentrations by hydrolysing them, thereby playing a role in antiviral defence. SAMHD1's catalytic activity depends on its tetrameric form, which is stabilised by the binding of GTP at allosteric site AL1 and a dNTP at site AL2. This study investigates whether Molecule X, an analogue of dNTPs, can promote SAMHD1 tetramerisation and, consequently, be hydrolysed by SAMHD1. Using size exclusion chromatography (SEC), SAMHD1's oligomeric states were analysed in the presence of GTP, dATP, and Molecule X. Results revealed that while GTP and dATP together facilitate the formation of the active tetrameric form of SAMHD1, GTP combined with Molecule X does not, indicating that Molecule X does not promote tetramerisation. However, preliminary NMR spectroscopy showed that SAMHD1 appears to slowly hydrolyse molecule X. Therefore, further research is required to confirm whether SAMHD1 hydrolyses molecule X and, if so, how it achieves this without inducing tetramerisation.

Keywords: SAMHD1, Nucleotide analogue, dNTPs, oligomerisation, allosteric regulation, antiviral

Introduction

Enzymes are biological catalysts used by living organisms to facilitate chemical reactions and are typically proteins. SAMHD1 is an enzyme whose substrates are deoxyribonucleotide triphosphates (dNTPs). dNTPs are the monomeric subunits of DNA.

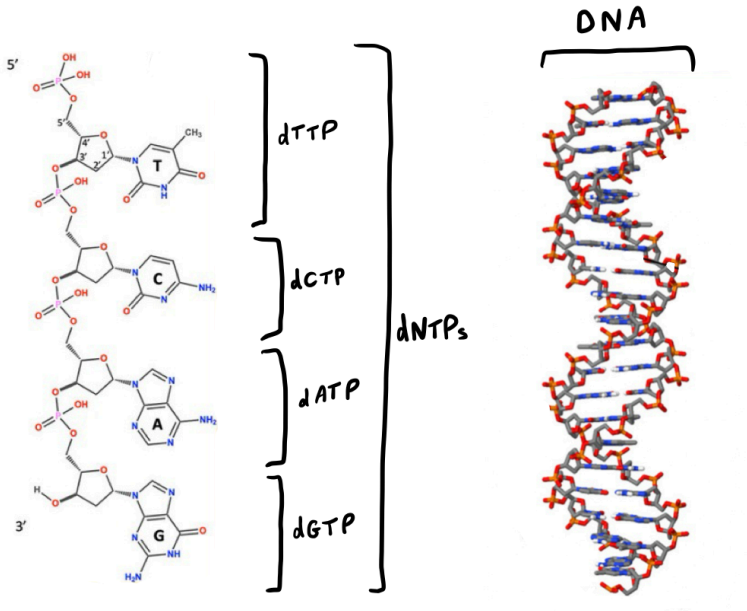

Figure 1: Structure of deoxyribonucleotide triphosphates (dNTPs) and DNA. The four types of dNTPs—dATP, dGTP, dCTP, and dTTP—each consist of a deoxyribose sugar, a phosphate group, and a nitrogenous base (adenine [A], guanine [G], cytosine [C], or thymine [T]). These dNTPs serve as the building blocks of DNA. The diagram on the right illustrates the double-helix structure of DNA formed by the polymerisation of these dNTPs. Figure adapted from [1].

SAMHD1 hydrolyses dNTPs into 2′-deoxynucleoside (dN) and Triphosphate (TP)[2]. This hydrolysis is critical for regulating the concentration of dNTPs in cells, and the catalytic activity of SAMHD1 is tightly linked to the cell cycle[3]. When a cell needs to replicate its genetic material, SAMHD1 activity is reduced, and therefore, dNTPs are available for synthesis[3]. However, during non-cell synthesis phases, SAMHD1 is active, limiting the number of dNTPs in the cell[3]. Retroviruses contain RNA and must turn their RNA genome into DNA through a process called reverse transcription before integrating their genetic material into the host genome. The virus uses the host cells' dNTPs to achieve this. Therefore, reducing the number of dNTPs available when the host cell is not replicating its own DNA gives retroviruses less opportunity to carry out reverse transcription. It is a mechanism that gives SAMHD1 its antiviral properties.

SAMHD1 is only catalytically active as a homotetramer[3]. The tetramerisation of SAMHD1 is regulated by its allosteric sites[3]. Allosteric regulation occurs when molecules other than the substrate bind to allosteric sites, causing conformational changes that affect enzyme activity. SAMHD1 has two allosteric sites, AL1 and AL2[3]. The binding of GTP at AL1 promotes a conformational change that facilitates the binding of additional nucleotides at AL2[3]. SAMHD1s second allosteric site, AL2, is specific for a dNTP and supports dimer-dimer interactions required for tetramerisation[3]. Only when AL1 and AL2 are occupied by their respective nucleotides does SAMHD1 form a stable and active tetramer that can hydrolyse dNTPs[3].

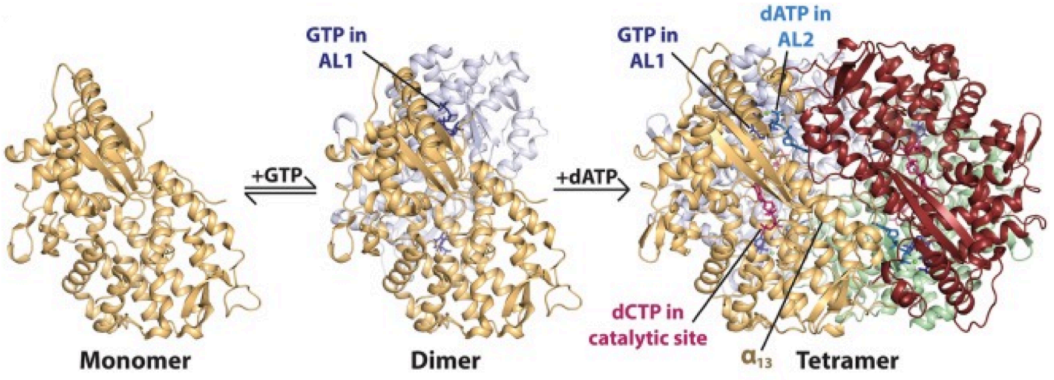

Figure 2: Structural transitions of SAMHD1 from monomer to active tetramer. The activation of SAMHD1 involves transitioning from a monomer to a tetramer, which is necessary for its catalytic activity. In the monomeric state, SAMHD1 is inactive. The binding of GTP at AL1 promotes dimer formation. When an additional nucleotide, such as dATP, binds to AL2, two dimers associate to form a homotetramer. In the tetrameric form, SAMHD1's catalytic site can hydrolyse dNTPs, as shown by the binding of dCTP. Figure adapted from [3]

Enzymes are highly specific to their substrates, only acting on specific molecules and their analogues. Molecule X is an analogue of dNTPs[4]. It is a chain terminator, meaning it stops the extension of a DNA chain during synthesis and, therefore, acts as an antiviral molecule itself[4]. Therefore, as SAMHD1 and molecule X both have antiviral mechanisms, it would make sense that they have evolved to exclude one another. So, despite being an analogue of SAMHD1's substrate dNTPs, molecule X should not be hydrolysed by SAMHD1.

The interaction between Molecule X and SAMHD1 was explored to investigate this hypothesis. Size exclusion chromatography (SEC) was utilised to examine the oligomeric states of SAMHD1 in the presence of different nucleotides. This experiment determines the ability of these molecules to promote the tetramerisation of SAMHD1 and, therefore, suggests whether molecule X interacts with SAMHD1 similarly to dNTPs.

Results and discussion

To investigate the oligomeric states of SAMHD1 in the presence of different nucleotide combinations, size exclusion chromatography (SEC) was performed on SAMHD1 (residues 109-626) under various conditions. The elution profiles of SAMHD1 alone and SAMHD1 incubated with GTP, GTP + dATP, and GTP + Molecule X are shown in Figure 3. GTP is included with dATP and Molecule X to ensure AL1 site filling is not the factor limiting tetramerisation.

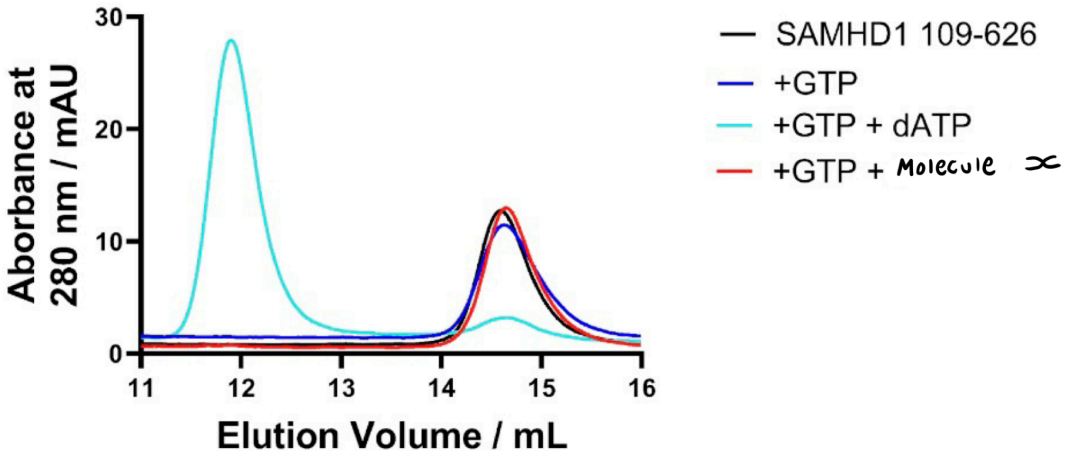

Figure 3: Size Exclusion Chromatography elution profile of SAMHD1's catalytic domain (residues 109-626) in the Presence of Different Nucleotides. SAMHD1 alone (black trace) shows a major peak corresponding to its dimeric form. In the presence of GTP alone (blue trace), a minimal shift in the elution profile is observed, suggesting no interaction with GTP. When GTP and dATP are combined (cyan trace), a prominent peak at ~12 mL indicates tetramerisation of SAMHD1. However, when Molecule X is present along with GTP (red trace), SAMHD1 fails to tetramerise, as evidenced by the absence of a peak at ~12 mL and the dominance of the dimeric form (~14.5 mL). This suggests that Molecule X does not promote the formation of the active tetrameric state of SAMHD1. Absorbance was monitored at 280 nm. Figure prepared by Rosella Pickney.

In the absence of nucleotides, SAMHD1 elutes as a single peak at an elution volume of ~14.5 mL, corresponding to the dimeric form. There is no significant shift in the elution profile upon adding GTP alone, indicating minimal structural change in oligomerisation. This supports the idea that while GTP binding at the AL1 site may cause some conformational change, it does not by itself stabilise the tetramer. However, the presence of both GTP and dATP results in a large peak at ~12 mL, consistent with the formation of the tetrameric form of SAMHD1. This confirms that combining GTP at AL1 and dATP at AL2 is necessary for SAMHD1 to oligomerise into its active tetrameric form. When Molecule X is substituted for dATP, SAMHD1 fails to form tetramers, as shown by the absence of the large peak at ~12 mL and the persistence of the dimeric peak at ~14.5 mL. These results indicate that Molecule X does not promote tetramerisation, implying that it either does not bind effectively to the AL2 site or does not induce the necessary conformational changes to drive tetramer formation.

Further characterisation using UV monitoring at 260 and 280 nm (Figure 4) confirmed that while dATP and dCTP facilitate SAMHD1 tetramerisation, Molecule X does not.

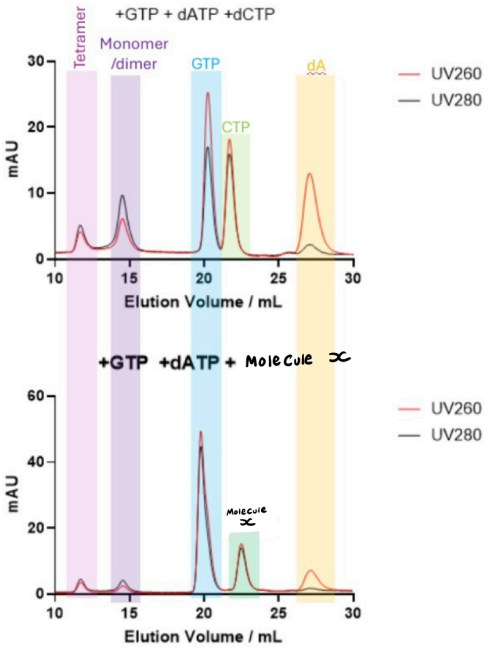

Figure 4: Size exclusion chromatograms showing the elution of SAMHD1 and nucleotides in the presence of different nucleotide combinations; absorbance was monitored at 260 nm (red trace) and 280 nm (black trace). The upper panel shows SAMHD1 incubated with GTP, dATP, and dCTP. A prominent tetramer peak is observed at ~12-14 mL, indicating tetramerisation of SAMHD1 in the presence of these nucleotides. Peaks corresponding to monomers/dimers (~15-18 mL) and free nucleotides (GTP, dCTP, dATP) are also visible at later elution volumes (~20-25 mL). The lower panel shows SAMHD1 incubated with GTP, dATP, and Molecule X. While tetramerisation is still present, the peak is reduced in intensity, suggesting that Molecule X is less effective at promoting tetramerisation than dCTP. A distinct peak for free Molecule X appears at ~20 mL, indicating poor affinity to SAMHD1. The results indicate that Molecule X does not significantly interact with SAMHD1 to stabilise its tetrameric form. Figure prepared by Rosella Pickney.

In the lower panel in Figure 4, a distinct peak for the free nucleotide analogue was detected at ~20 mL, indicating that the analogue did not bind effectively to SAMHD1 and was eluted on its own, unbound. Despite being an analogue of SAMHD1's natural substrates, these results support the hypothesis that molecule X does not promote the enzyme's active form. This lack of interaction could result from structural differences between Molecule X and natural dNTPs, preventing proper binding to the AL2 site and thus failing to stabilise the active tetrameric state of SAMHD1.

Figure 5 illustrates the time-dependent cleavage activity of SAMHD1 with its natural substrate, dCTP, and the nucleotide analogue, molecule X, as monitored using NMR spectroscopy.

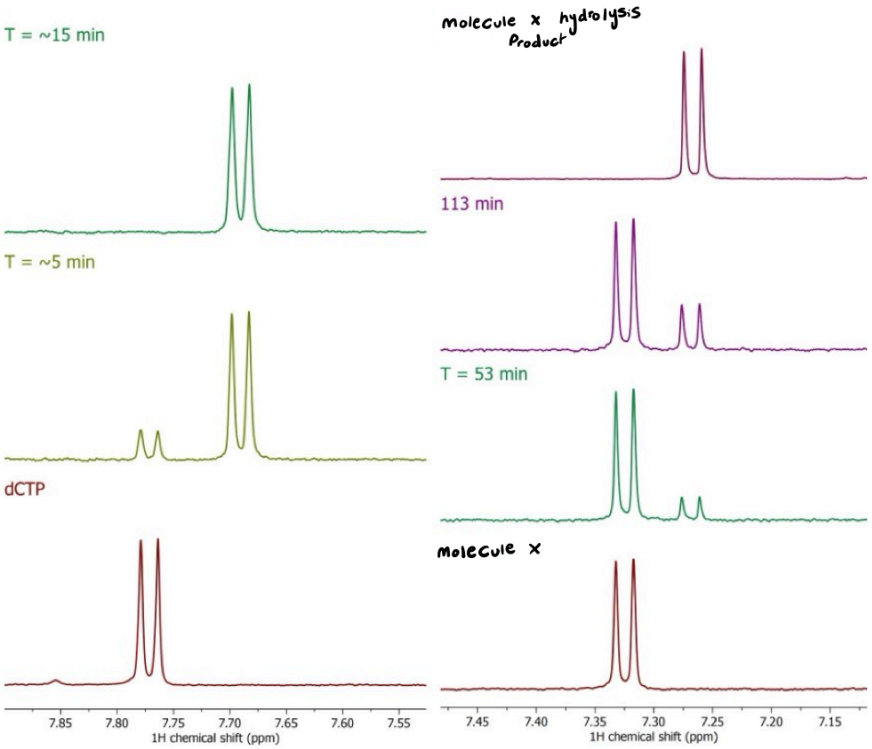

Figure 5: NMR spectra of SAMHD1 incubated with dCTP (left panel) and molecule X (right panel) over time. The bottom spectra in each panel represent the pure substrates, dCTP and molecule X, respectively. The spectra above show the time course of reactions after the addition of SAMHD1. For dCTP, cleavage into dC is observed rapidly, with significant accumulation within 15 minutes. For molecule, the cleavage occurs much more slowly, with detectable formation only after 53 and 113 minutes. Figure prepared by Rosella Pickney.

The NMR data reveals a differential in the processing of dCTP and molecule X by SAMHD1. As expected, SAMHD1 effectively cleaves dCTP, with a new peak corresponding to the product, dC, appearing shortly after the enzyme is added. Unexpectedly, SAMHD1 also hydrolyses molecule X, albeit at a significantly slower rate, as evidenced by the emergence of a peak after prolonged incubation times (53 and 113 minutes). The slow and inefficient cleavage of molecule X suggests it is a poor substrate for SAMHD1, yet its ability to be processed at all was unanticipated.

Materials and methods

Bacterial Transformation

The SAMHD1 gene (residues 109-626) was cloned into a pET52b+ vector, which confers antibiotic ampicillin resistance. The vector was transformed into *E.coli* Rosetta 2 cells. These cells carry the pRARE2 plasmid, which supplies tRNAs for 7 rare codons often required for human protein expression in bacterial systems. The plasmid is also antibiotic chloramphenicol resistant.

Bacterial culture

These cells were then plated on LB agar containing ampicillin and chloramphenicol. As competent cells would be both ampicillin and chloramphenicol-resistant, colonies grew on the agar. Competent cells were transferred to a Terrific broth medium and then incubated to encourage the proliferation of bacteria.

SAMHD1 expression

To control protein expression, the SAMHD1 gene is integrated into the lac operon system of the bacterial expression vector. This system allows the regulation of protein synthesis through the addition of Isopropyl β-D-1-thiogalactopyranoside (IPTG), a molecular mimic of allolactose. IPTG binds to the lac repressor protein, triggering its release from the operator region of the lac operon. This removal of the repressor permits RNA polymerase to access the promoter and initiate transcription of the SAMHD1 gene, thereby inducing protein expression in *E. coli*.

SAMHD1 Purification

Cultures are then centrifuged, and the pellet containing bacterial cells with expressed SAMHD1 protein is harvested. Cells are lysed by sonication to release all cellular proteins. The cells are centrifuged again to separate soluble proteins from insoluble debris (such as cell walls and other cellular particles), and the supernatant, which contains soluble proteins, including the target protein (SAMHD1), is collected.

The SAMHD1 protein was engineered to include a Strep-tag, a short peptide sequence that enables affinity purification using a StrepTactin column. The Strep-tag binds specifically to the StrepTactin resin, enabling selective purification of the tagged SAMHD1 protein. A protease cleavage site between the SAMHD1 protein and the strep-tag is also included. This allows for the elution of purified SAMHD1 when protease is added to the column.

Size Exclusion Chromatography

Size exclusion chromatography (SEC) was used to investigate the oligomeric states of SAMHD1 under various conditions. SEC is a chromatographic method that separates molecules based on their size by passing them through a column packed with porous beads. Larger molecules elute earlier as they are excluded from entering the pores, while smaller molecules penetrate the beads and elute later.

For this study, SAMHD1 and its complexes were separated using a Superdex 200 26/60 column, equilibrated with SEC buffer. The samples were loaded onto the column, and their elution profiles were monitored at 260nm and 280nm. The tetrameric form of SAMHD1, being the largest oligomer, eluted first, followed by smaller oligomeric forms such as dimers and monomers. This allowed for the assessment of SAMHD1's oligomeric state in the presence of different nucleotide combinations.

Conclusions

In conclusion, molecule X does not induce tetramerisation of SAMHD1; however, it appears to be slowly hydrolysed by SAMHD1 in preliminary NMR experiments. Further research should focus on confirming whether SAMHD1 hydrolyses molecule X using enzymatic assays and investigating the interaction kinetics to determine the chemical reasons for the lack of tetramerisation. Understanding this dynamic could reveal how SAMHD1 discriminates between its natural substrates and analogues, potentially informing the development of antiviral strategies utilising this mechanism.

References

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