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Crystallographic studies of the complex of human HINT1 protein with a non-hydrolyzable analog of Ap_4A

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ABSTRACT

Histidine triad nucleotide-binding protein 1 (HINT1) represents the most ancient and widespread branch in the histidine triad proteins superfamily. HINT1 plays an important role in various biological processes, and it has been found in many species. Here, we report the first structure (at a 2.34 Å resolution) of a complex of human HINT1 with a non-hydrolyzable analog of an Ap_4A dinucleotide, containing bis-phosphorothioated glycerol mimicking a polyphosphate chain, obtained from a primitive monoclinic space group $P2_1$ crystal. In addition, the apo form of hHINT1 at the space group $P2_1$ refined to 1.92 Å is reported for comparative studies.

1. Introduction

Histidine triad nucleotide-binding protein 1 (HINT1) belongs to a branch of the histidine triad (HIT) proteins superfamily, primarily consisting of mono- and dinucleotide hydrolases and nucleotide transferases. They have a characteristic C-terminal active site motif HXHXHXX, where X is a hydrophobic residue [1]. HINT proteins are the most conserved members of the HIT superfamily with nucleotide hydrolase activity. HINT protein homologs are present in many organism kingdoms, including metazoan [2–4], plant [5], fungus [6], and bacterial [7,8]. Their fundamental role in cell metabolism is suggested by strongly conserved amino acids sequences. HINT1 is expressed in multiple tissues and is present in nuclei and cytoplasm, but its function is still poorly defined. It is involved in transcription regulation [9–13] and cell cycle control [14]. In mammals, HINT1 modulates apoptosis in cancer cells, and is a potential tumor suppressor [11,13,15–21]. Recent studies have shown an important role of HINT1 as a platform protein involved in formation of the G-protein coupled receptors (GPCRs)—MOR (μ-opioid receptor) and CNR1 (type 1 cannabinoid receptor) [22,23].

The first HINT1 crystal structure was published in 1996 [24], and the protein was initially described as an inhibitor of protein kinase C [25], and further, as PKCI (protein kinase C-interacting) [26]. The name "HINT" emerged from the structural analysis [27]. At present, there are eighteen HINT1 structures in PDB: ten of them are of human [24,28–30], six of rabbit [27,31,32] and two of *Escherichia coli* origin [8]. The deposited structures display HINT either in the apo state [24] or as complexes with selected ligands—either substrate analogs or products of their hydrolysis [8,28,31]. Up to now, the highest registered data resolution for hHINT1 was 1.38 Å (PDB code 3tw2 [30]).

In vitro studies indicate that human HINT1 binds various nucleotides, e.g., adenosine-5′-monophosphate (AMP), adenosine-5′-diphosphate (ADP), and diadenosine polyphosphates: 5′,5″-diadenosine triphosphate (Ap_3A) and 5′,5″-diadenosine tetraphosphate (Ap_4A) [24]. Rabbit HINT1 (rHINT1) binds also certain purine nucleosides and nucleotides [27]. HINT proteins exert phosphoramidase activity towards adenosine-5′-*O*-phosphoramidate (AMP-NH_2) [2]. Also, lysyl-adenylate (generated by lysyl-tRNA synthetase (LysRS)) is suggested to be a substrate for HINT1 hydrolase [33,34]. HINT1 assisted hydrolysis of the P—N bonds in nucleoside phosphoramidates was demonstrated by Murakami et al. [35]. Rabbit HINT1 also desulfurates 5′-*O*-phosphorothioylated nucleosides [36,37].

Dinucleoside 5′,5″-P^1,P^n-polyphosphates (also called Np_nN's, where N and N′ are 5′-*O*-nucleosidyl residues and *n* is the number of phosphate residues in a polyphosphate linkage), occur in prokaryotes and eukaryotes [38]. Most abundant are dinucleoside tri- and tetra-phosphates. Their biological roles remain fairly obscure, with some data suggesting a variety of possible functions [39]. Several ligases and transferases synthesize Np_nN's, while various enzymes control their degradation [40]. Numerous analogs have been tested

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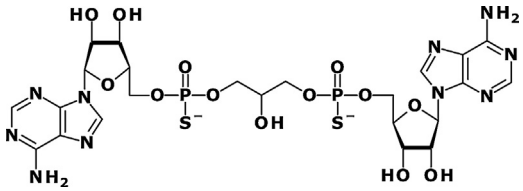

Fig. 1. Chemical structure of JB419 compound.

as alternative substrates and/or inhibitors of HIT proteins. In our earlier studies, the Ap_4A analogs were synthesized [41] and tested as inhibitors of FHIT (fragile histidine triad) protein, which is another member of the HIT family [42]. One of the most potent inhibitors was 1,3-bis(adenosine-5′-*O*-phosphorothioyl) glycerol, denoted JB419 (Fig. 1) [42,43].

In the present manuscript, we report the structure of human HINT1 in a complex with JB419 at a 2.34 Å resolution, obtained from a monoclinic crystal form. Suitable crystals were obtained by the vapor diffusion method in a hanging drop variant at 281 K. The ligand affected the arrangement of the crystal asymmetric unit, compared to the crystals of apo HINT1 obtained in the same space group. The presented HINT1/JB419 complex structure is the first one of the HINT1 protein in a complex with a ligand other than a nucleoside, a mononucleotide or their analog. In addition, for comparative studies, the apo HINT1 structure crystallized in $P2_1$ space group is also presented.

2. Materials and methods

2.1. Materials

2.1.1. Cloning, expression and purification of hHINT1

Human HINT1 protein was expressed and purified in *E. coli* BL21 strain using pSGA02-*hHINT1* plasmid as previously described for rabbit HINT1 [44]. A one-step purification was performed using AMP-agarose (Sigma) affinity chromatography. The homogenous protein preparation was dialyzed against a buffer containing 20 m*M* Tris-HCl (pH 7.5) and 150 m*M* NaCl, concentrated to the protein concentration of 10 mg ml^{-1}, frozen in liquid nitrogen and stored at −80 °C.

2.1.2. Synthesis of a substrate analog JB419

JB419 was synthesized as previously described [41]. Shortly, our approach was based on phosphorothioylation of glycerol with 2-chloro-1,3,2-oxathiaphospholane in the presence of elemental sulfur in pyridine, followed by a ring-opening reaction of the resulting glycerol-1,3-*O*,*O*-bis-(2-thio-1,3,2-oxathiaphospholane) with N^6,N^6,$O^{2'}$,$O^{3'}$-tetrabenzoyladenosine in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). After alkaline removal of the benzoyl protecting groups and chromatographic purification, the resulting product (a diastereomeric mixture) was characterized by FAB MS and ^{31}P NMR methods.

2.2. Methods

2.2.1. Preparation and crystallization of hHINT1/JB419 complex and apo hHINT1

The hHINT1/JB419 complex was crystallized by a standard hanging drop variant of the vapor diffusion method, using a mixture of 6 mg ml^{-1} protein solution with 8-fold molar excess of JB419. Initial crystallization conditions were similar to those previously used for hHINT1, and were a starting point for optimization. Finally, the precipitant solution contained 0.1 M sodium cacodylate buffer (pH 5.5), and 19% w/v PEG4000. Aliquots of 1.5 μl of the protein/ligand solution were mixed with 1.5 μl of the precipitant solution and the crystallization drops were hanged above 1 ml of the precipitant solution. Crystals (in the shape of plates of typical dimensions 0.8 × 0.4 × 0.1 mm) were obtained after 48–72 h storage at 281 K.

Apo hHINT1 was also crystallized in the $P2_1$ space group, in contrast to the crystals typically obtained in $C2$ or $P4_32_12$ space groups. For crystallization, a 6 mg ml^{-1} protein solution and a precipitant solution containing 0.1 M sodium cacodylate buffer pH 6.8, and 18% w/v PEG4000 were used. Aliquots of 1.5 μl of the protein solution were mixed with 1.5 μl of the precipitant solution and the crystallization drops were hanged above 1 ml of the precipitant

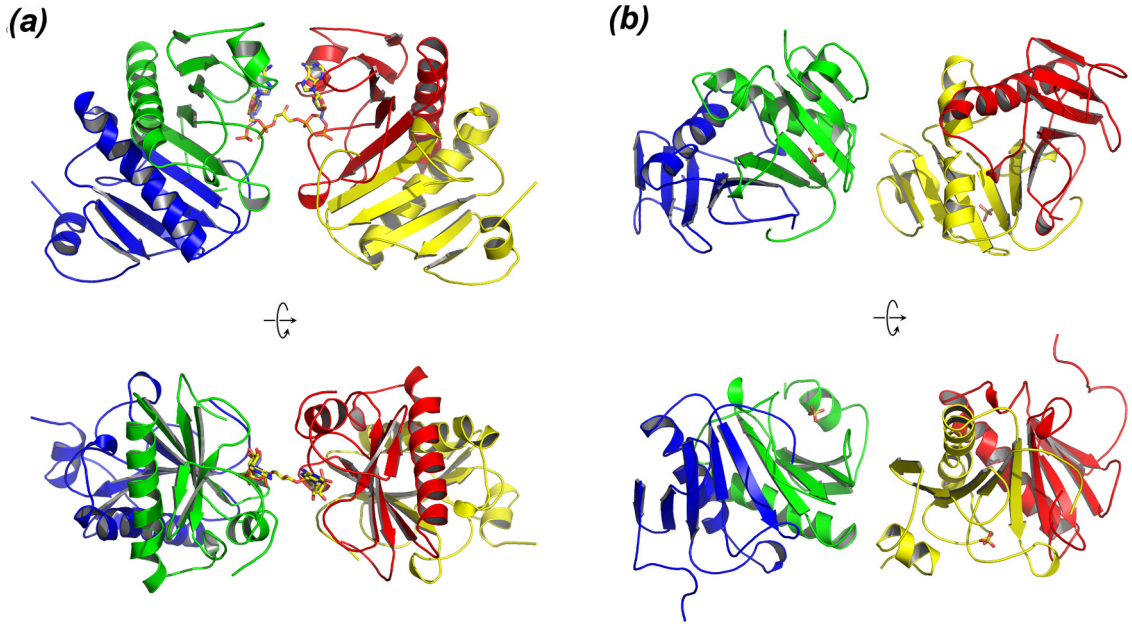

Fig. 2. Cartoon representation (based on PDB entry 4zkl and 4zkv) of overall structures of (a) the hHINT1/JB419 complex and (b) the apo hHINT1 in. Each of the protein monomer (A, B, C, and D) is shown in a different color scheme. The binding site location is indicated by the bound JB419 and AMP molecules (shown in a stick representation). Secondary structure elements were assigned with *DSSP* [56]. The views after 90° rotation are shown at the bottom.