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Defects in DNA degradation revealed in crystal structures of TREX1 exonuclease mutations linked to autoimmune disease

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ABSTRACT

Mutations within the human TREX1 3′ exonuclease are associated with Aicardi-Goutières Syndrome (AGS) and familial chilblain lupus (FCL). Both AGS and FCL are autoimmune diseases that result in increased levels of interferon alpha and circulating antibodies to DNA. TREX1 is a member of the endoplasmic reticulum (ER)-associated SET complex and participates in granzyme A-mediated cell death to degrade nicked genomic DNA. The loss of TREX1 activity may result in the accumulation of double-stranded DNA (dsDNA) degradation intermediates that trigger autoimmune activation. The X-ray crystal structures of the TREX1 wt apoprotein, the dominant D200H, D200N and D18N homodimer mutants derived from AGS and FCL patients, as well as the recessive V201D homodimer mutant have been determined. The structures of the D200H and D200N mutant proteins reveal the enzyme has lost coordination of one of the active site metals, and the catalytic histidine (H195) is trapped in a conformation pointing away from the active site. The TREX1 D18N and V201D mutants are able to bind both metals in the active site, but with inter-metal distances that are larger than optimal for catalysis. Additionally, all of the mutant structures reveal a reduced mobility in the catalytic histidine, providing further explanation for the loss of catalytic activity. The structures of the mutant TREX1 proteins provide insight into the dysfunction relating to human disease. Additionally, the TREX1 apoprotein structure together with the previously determined wild type substrate and product structures allow us to propose a distinct mechanism for the TREX1 exonuclease.

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1. Introduction

The TREX1 3′ exonuclease is a member of the DnaQ family of enzymes, and catalyzes the major 3′ exonuclease activity in mammalian cells [1,2]. The exact cellular role of TREX1 remains unclear, but several potential functions have been identified. These include degradation of nicked genomic DNA during granzyme A cell death [3], elimination of single-stranded DNA (ssDNA) derived from endogenous retroelements as a part of the interferon-stimulatory DNA response [4], and the disposal of aberrant ssDNA replication intermediates [5,6]. TREX1 interacts with the SET protein as a member of the endoplasmic reticulum (ER)-associated SET complex [3]. The 440 kDa SET complex contains proteins such as the endonuclease NM23H1, the apurinic endonuclease APE1, and the DNA binding protein HMG-2. During granzyme A-mediated cell death, the serine protease granzyme A enters a target cell and proteolyses certain components of the complex, which results in the translocation of the SET complex into the nucleus. Subsequently, the NM23H1 nuclease generates single-stranded nicks in the genomic DNA. TREX1 binds the 3′ termini generated at these nicks and fully degrades the DNA, ensuring cell death [3]. It has been proposed that this TREX1 function is important to avoid the accumulation of host DNA after cell death that could trigger an autoimmune response. Alternatively, there is evidence that TREX1 acts as a negative regulator of the interferon stimulatory DNA response pathway by degrading single- and double-stranded DNA (ssDNA, dsDNA) resulting from replication of endogenous retroelements or DNA repair intermediates [4,6]. Removal of this cytosolic DNA prevents a potent cell-intrinsic antiviral response activated through cytosolic DNA receptors.

Genetic studies have linked mutations in the TREX1 gene with a spectrum of autoimmune diseases including Aicardi-Goutières syndrome (AGS), familial chilblain lupus (FCL), systemic lupus erythematosus (SLE), and retinal vasculopathy and cerebral leukodystrophy (RVCL) [7–14]. Additionally, TREX1 deficient mice develop inflammatory myocarditis, strengthening the association with autoimmune disease [15]. The sites of mutations causing autoimmune diseases in humans are located throughout the TREX1 gene; affecting areas of the protein including the active site, the dimer interface, and the C-terminal domain. Mutations within the

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TREX1 active site have been linked to both AGS and FCL. AGS is a genetically determined encephalopathy exhibiting recessive inheritance, although isolated cases of dominant AGS have been identified. The symptoms of AGS mimic congenital viral infection without evidence of an infectious agent [16–18]. These patients exhibit a severe phenotype involving the calcification of basal ganglia and white matter in the brain that negatively impacts motor and social development [18–20]. FCL is a dominant form of lupus erythematosus [9,11,21], which shares significant phenotypic overlap with AGS including increased levels of interferon alpha and circulating antinuclear antibodies to dsDNA or ssDNA, suggesting a common pathogenesis.

The TREX1 protein is a stable homodimer in solution, and the crystal structure revealed that the active sites are positioned at opposite outer edges of the same face of the dimer [22,23]. The TREX1 protein contains four highly conserved acidic residues (DEDD) within the active site that are characteristic of the DnaQ family of exonucleases. These residues are required for coordinating two Mg^{2+} ions that are necessary for catalysis. The presence of a conserved histidine residue in the TREX1 active site places the protein within the DEDDh subgroup of the DnaQ family. The histidine is thought to contribute to deprotonation of a water molecule and to promoting nucleophilic attack on the scissile phosphate during catalysis [24–27]. The TREX1 protein has several additional distinct structural features that likely contribute to its cellular function. A polyproline type II (PPII) helix is positioned adjacent to the active site and dimer interface in each TREX1 protomer. The PPII helix has a putative role in mediating contacts with other proteins in the cell, and may be important for its interaction with the SET complex. The TREX1 protein also has an extended C-terminal region that is involved in the sub-cellular localization of TREX1 on the cytosolic side of the endoplasmic reticulum [3,12]. Finally, a flexible loop that is proposed in DNA binding is located adjacent to each active site [26,27].

Multiple mechanisms of dysfunction underlie the observed clinical phenotypes in patients with TREX1 mutations, reflective of the fact that the mutations result in a broad range of catalytic activities, yet all result in similar human pathologies. Our previous biochemical data has begun to provide insight into the disease etiology for dominant TREX1-mediated FCL and AGS, but the mechanism of recessive AGS remains elusive. We have proposed a mechanism for biological dysfunction of dominant TREX1 mutants in which competitive binding of DNA nicks by the catalytically inactive dominant mutants precluding access by active TREX1 [28,29]. In order to better understand the effects of disease causing mutations on the structure and function of the TREX1 enzyme, we have determined the X-ray crystal structures of four mutant TREX1 proteins identified in patients with autoimmune disease, as well as the structure of the wild type TREX1 apoprotein. The D18N, D200N and D200H, TREX1 proteins are dominant mutants associated with FCL and AGS. The V201D mutant TREX1 is a recessive AGS mutation. All of these mutations are located in or directly adjacent to the TREX1 active site and display a spectrum of catalytic activities ranging from 25% of wild type activity to virtually inactive enzyme. The role of the D18 and D200 residues is to coordinate the two divalent metal ion cofactors necessary for catalysis. The structures of the mutant TREX1 proteins demonstrate a fundamental role for conformational movement of the catalytic histidine during nucleic acid hydrolysis. This reveals unique characteristics of two metal ion catalysis in the context of the DEDDh subgroup of the DnaQ family of exonucleases that rationalizes substrate trapping in FCL and dominant AGS. The structure of the TREX1 apoprotein, reveals conformational differences in the active site dependent upon the presence or absence of substrate. Additionally, in the structure of the D200H TREX1 mutant we observe an alternate conformation of the conserved catalytic residue, E20, which forms an interaction with an arginine from the opposing protomer. These data provide new insight into possible substrate release during the catalytic cycle, and for the first time provide a rationale for the unique dimeric architecture of the TREX1 protein.

2. Materials and methods

2.1. Protein expression and purification

The mouse TREX1 enzymes used for crystallization contain the catalytic core residues (1–242) of the protein. This C-terminal truncation was expressed as a fusion with an N-terminal polyhistidine sequence followed by maltose binding protein (MBP) as described previously [22]. Briefly, the expression vector containing the MBP–TREX1 fusion was transformed into BL21*(DE3) Rosetta II cells (Novagen) for overexpression. The cells were grown to an $A_{600} = 0.5$ and induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 10 min at 37 °C and quickly cooled on ice to 16 °C. Cells were allowed to grow for 18 h at 16 °C. The MBP–TREX1 fusion was bound to amylose resin, washed thoroughly, and cleaved overnight by incubation with PreScission Protease (GE Life Sciences) at 4 °C to remove the polyhistidine tag and MBP protein. After cleavage, the mTREX1 protein was collected in the column flow-through and dialyzed against 50 mM Tris–HCl pH 7.5, 50 mM NaCl, 10% glycerol and 1 mM EDTA and purified to homogeneity using phosphocellulose chromatography.

2.2. Protein crystallization and X-ray data collection

The TREX1 mutant proteins and the wild type TREX1 apoprotein were crystallized using the sitting drop vapor diffusion technique. All TREX1 proteins were dialyzed into 20 mM MES (pH 6.5), 50 mM NaCl. Substrate complex was formed by incubating the protein with a four-nucleotide ssDNA (5′-GACG, purchased from Operon) in a molar ratio of 1:2 and 5 mM calcium chloride (5 mM magnesium chloride in the case of the D200N mutant). TCEP–HCl pH 8.0 was added to a final concentration of 1 mM to each protein solution prior to placing in the crystallization tray. 2 μl protein complex at 5 mg/ml TREX1 was mixed with an equal volume of reservoir solution and placed on a bridge above 500 μl of the reservoir solution. Optimized crystals of the wild type TREX1 apoprotein grew in 0.25 M tri-sodium citrate dihydrate and 20% PEG 3350. Optimized crystals of the D18N complex were obtained by microseeding into 0.15 M MES pH 6.5, 19% PEG 4000 and 10% ethylene glycol and grown at 15 °C. Crystals of the D200N mutant complex were obtained by microseeding into 0.1 M MES pH 5.5, 0.075 M NaCl, 12% PEG 3350 and 5% 1,4-butanediol and grown at 15 °C. The D200H complex was obtained by microseeding into 0.1 M MES pH 6.0, 16% PEG 4000, 2% 1,4-butanediol and grown at 30 °C. Crystals of the V201D protein in complex with ssDNA were grown at 30 °C in 16% PEG 3350, 0.1 M NaI and 5% 1,4-butanediol. All crystals grew within 1 week. Prior to data collection all crystals were dipped into reservoir solution containing 20% 1,4-butanediol or (for D18N) 20% glycerol in preparation for cryo-cooling. Crystals were mounted on a nylon loop and flash cooled to 100 K in a stream of liquid nitrogen.

2.3. Phasing and refinement

The X-ray data were collected using CuKα radiation on a MicroMax 007 generator and a Saturn 92 CCD detector (Rigaku). Intensity data were processed using the programs d*TREK or HKL2000 [30,31]. The D18N, D200N, and D200H TREX1 mutants in complex with ssDNA belong to the $P2_1$ spacegroup. The TREX1 V201D–ssDNA complex and the wild type TREX1 apoprotein belong

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