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Crystal structure of HslV from *Trypanosoma brucei*

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1 Introduction

The heat shock protein complex, HslVU, is a simple model system of the eukaryotic 26S proteasome. In many bacteria, heat shock locus V (HslV) functions as a protease with its activator heat shock locusU (HslU), which is an unfoldase driven by ATP hydrolysis. In *Trypanosoma brucei*, a protozoan parasite that causes human sleeping sickness in Africa, HslV is localized in the mitochondria, where it has a novel function in regulating mitochondrial DNA replication. Although structures of the prokaryotic and archaeal HslV protease have been studied previously [1,2], little is known regarding its eukaryotic counterpart. In contrast to the prokaryotic system, *T. brucei* possesses two potential HslU molecules, and it has been found that only one of them activates HslV. We reported the crystal structure of a eukaryotic HslV from *T. brucei* (TbHslV), determined at 2.4 Å resolution [3].

2 Experiment

TbHslV was expressed as His-tagged form and purified by nickel-NTA affinity chromatography followed by anion exchange and gel filtration chromatography. The TbHslV was crystallized using the hanging drop or sitting-drop vapor-diffusion method at 22 ºC. The Form-I crystal belongs to a monoclinic space group $P2_1$ with unit cell parameters of a=100.9 Å, b=107.0 Å, c=132.8 Å, and β=104.3º. The crystal Form-II was obtained in the orthorhombic space group I222, with cell parameters of a=105.9 Å, b=111.5 Å, c=117.2 Å, and α=β=γ=90°. Diffraction data were collected at the NW12 beamline of the Photon Factory, Tsukuba, Japan, by using an ADSC quantum charge-coupled device detector. A total of 180 images were collected with 1º oscillation, and each image was exposed for 0.8 sec. The diffraction data were processed and scaled using the HKL2000 software package. Phases were obtained by molecular replacement with the program MOLREP in the CCP4 program suite. A previously determined structure of HslV from *E. coli* was used as a search model [1]. The initial model was rebuilt and refined using standard protocols in COOT, PHENIX, and REFMAC until the *R*-factor was converged.

3 Results

The general structural features of TbHslV are well conserved with a dodecamer of 2 stacked hexameric rings containing an axial entrance pore (Fig. 1A & 1B), the proteasomal β-subunit folding of its monomer (Fig. 1C). The monomeric subunit of TbV is comprised of 4 α-helices, 11 β-strands, and connecting loops. The structural results show that polar interactions including hydrogen bond and salt bridges are dominant during formation of the hexameric ring, while hydrophobic interactions contribute to the donut-shape TbHslV dodecamer (Fig. 1A).

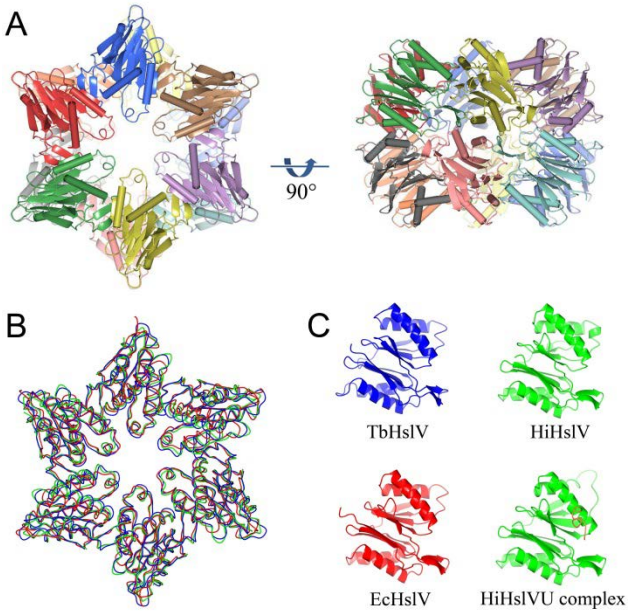

Fig. 1: Structure of TbHslV and comparison with other HslVs. (A) Ribbon diagram of dodecameric TbHslV viewed along a 6-fold molecular symmetry axis (Left). A side view of the TbHslV showing twofold molecule symmetry at the center of the molecule (Right). (B) Superposition of TbHslV and other HslVs viewed along a 6-fold axis. For clarity, only the upper hexameric ring is shown. The colors for each molecule are blue, red, and green for TbHslV, EcHslV, and HiHslV, respectively. (C) Comparison of monomeric subunits among TbHslV, EcHslV, and HiHslV (and HiHslV in complex with HiHslU) with colored as panel B. The bound C-terminal segment of HiHslU is shown as a red trace (HiHslVU complex).

References

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