Structure and function of the proteasome activator PA28 of the malaria parasite Plasmodium falciparum

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The proteasome is a multi-subunit enzyme complex that is responsible for most of the non-lysosomal proteolysis in eukaryotic cells, underpinning proteostasis and regulating key processes such as the cell cycle. The 20S proteasome core is comprised of two heptameric rings of β-subunits, containing the catalytic sites, sandwiched by two heptameric α-rings. Substrate access to the proteasome catalytic sites is restricted by N-terminal sequences of the alpha-subunits (1). The activity of the 20S proteasome is regulated by protein complexes that bind to one or both ends of the proteasome. One class of such regulators is PA28 proteasome activation complex (also called 11S or REG).

Here we recombinantly expressed and purified an orthologue of the PA28 regulator (*Pf* PA28) from *Plasmodium falciparum.* The structure of *Pf*PA28 was solved at 3.1 Å by X-ray crystallography, revealing a heptameric bell-shaped structure. We also purified *Pf*20S proteasome from parasite cultures and showed by Native-PAGE that *Pf*PA28 readily forms single- and double-capped complexes with *Pf*20S. Moreover, we demonstrated the stimulatory effects of *Pf*PA28 on *Pf*20S activity using fluorogenic peptide substrates. We next structurally characterized the *Pf*PA28-*Pf*20S complex using cryo-EM. Samples (500 μg/mL) were prepared on quantifoil grids and vitrified before collection of data using a Talos Arctica with a K2 camera. The activation loop (between helices 2 and 3 in each *Pf*PA28 monomer) plays a primary role in the interaction with the α-rings of *Pf*20S. In addition, the flexible C-terminal tails of *Pf*PA28 insert into the hydrophobic pockets between α-subunits and become ordered upon complex formation. The interaction induces conformational changes in α-subunits consistent with opening of the α-annulus, to allow substrate entry to the proteasome catalytic chamber. Long disordered loops (>50 residues between helices 1 and 2 in each monomer) at the distal side of the *Pf*PA28 heptamer are evident in Small Angle X-ray Scattering (SAXS) data and observed in cryoEM class averages, with parts of this loop resolved in some subunits of the crystal structure. We propose that these loops form an entropic brush that controls the entry of substrates to the highly basic entry pore.

1. Groll M*, et al.* (1997) Structure of 20S proteasome from yeast at 2.4 A resolution. *Nature* 386(6624):463-471.