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Research review paper

Hookworm SCP/TAPS protein structure—A key to understanding host–parasite interactions and developing new interventions☆,☆☆

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ABSTRACT

SCP/TAPS proteins are a diverse family of molecules in eukaryotes, including parasites. Despite their abundant occurrence in parasite secretomes, very little is known about their functions in parasitic nematodes, including blood-feeding hookworms. Current information indicates that SCP/TAPS proteins (called *Ancylostoma*-secreted proteins, ASPs) of the canine hookworm, *Ancylostoma caninum*, represent at least three distinct groups of proteins. This information, combined with comparative modelling, indicates that all known ASPs have an equatorial groove that binds extended structures, such as peptides or glycans. To elucidate structure–function relationships, we explored the three-dimensional crystal structure of an ASP (called *Ac*-ASP-7), which is highly up-regulated in expression in the transition of *A. caninum* larvae from a free-living to a parasitic stage. The topology of the N-terminal domain is consistent with pathogenesis-related proteins, and the C-terminal extension that resembles the fold of the Hinge domain. By anomalous diffraction, we identified a new metal binding site in the C-terminal extension of the protein. *Ac*-ASP-7 is in a monomer–dimer equilibrium, and crystal-packing analysis identified a dimeric structure which might resemble the homo-dimer in solution. The dimer interaction interface includes a novel binding site for divalent metal ions, and is proposed to serve as a binding site for proteins involved in the parasite–host interplay at the molecular level. Understanding this interplay and the integration of structural and functional data could lead to the design of new approaches for the control of parasitic diseases, with biotechnological outcomes.

Contents

☆ *Note:* "Hinge" denotes the C-terminal moiety found in many SCP/TAPS proteins; "hinge" is used to describe a flexible region linking two domains or moieties.
☆☆ Dedicated to Robert Huber on the occasion of his 75th birthday.

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1. Introduction

Soil-transmitted helminths (geohelminths) are causative agents of neglected tropical diseases (NTDs), mainly in developing countries (Hotez et al., 2007). In particular, blood-feeding hookworms, including species of *Necator* and *Ancylostoma* (Nematoda), infect ~740 million people in rural areas of the tropics and subtropics (de Silva et al., 2003), causing an estimated disease burden of 22 million disability-adjusted life years (Hotez et al., 2006). Hookworms infect humans by direct transmission of infective, third-stage (L3) larvae from the environment. These larvae usually penetrate the human skin and, following migration via the circulatory system and lungs, develop through to adults, which reside in the duodenum. The dioecious adults attach to the intestinal mucosa, rupture capillaries and feed on blood. Consequently, hookworm disease is characterized by anaemia, and often leads to physical and mental retardation and sometimes death in children and adverse maternal–fetal outcomes (Bethony et al., 2006; Loukas et al., 2006). Although anthelmintic drugs are used to reduce the burden of disease in a range of countries, mass treatment programs carry a significant risk of inducing drug resistance in hookworm populations (Cantacessi et al., 2010). Therefore, in recent years, there has been a major focus on developing new approaches to control hookworm disease, based on improved knowledge of the pathogen, the pathogen–host interactions and the disease at the molecular and immunological levels. Often, the canine hookworm, *Ancylostoma caninum*, has been used as a model for this purpose.

Various studies have implicated activation-associated proteins (ASPs) in an immunomodulatory function during a hookworms' invasion of the host, migration through tissues, attachment to the intestinal wall and blood-feeding (reviewed by (Cantacessi et al., 2009)). These are amongst the ten most abundant groups of proteins in hookworms (Cantacessi et al., 2009). Moreover, during the transition of *A. caninum* from a free-living to parasitic L3, 17 of the 30 most highly up-regulated mRNAs encoded members of the ASP family (Datu et al., 2008). ASPs belong to a large group of proteins, the 'sperm-coating protein (SCP)-like extracellular proteins', also called SCP/Tpx-1/Ag5/PR-1/Sc7 (=SCP/TAPS; Pfam accession number no. PF00188), characterized by the presence of a single or double 'SCP-like extracellular domain' (InterPro: IPR014044). In *A. caninum*, double and single SCP-domain ASPs, designated *Ac*-ASP-1 and *Ac*-ASP-2, respectively, are secreted in response to host-specific signals during the infection process (Bethony et al., 2005 ; Hawdon et al., 1996). ASPs also dominate the excretory/secretory (ES) proteins released by the blood-feeding adult stage hookworms (Mulvenna et al., 2009). Homologues of *Ac*-ASP-1 and *Ac*-ASP-2 have been identified also in the L3 stage of *N. americanus* (Goud et al., 2004, 2005; Zhan et al., 1999). Results from crystallography (Asojo et al., 2005), combined with the observation that one of these ASPs (*Na*-ASP-2) induces neutrophil and monocyte migration (Bower et al., 2008), suggest that this molecule has a role as an antagonistic ligand of complement receptor 3 (CR3) and alters the immune cascade by preventing the binding of chemotaxin (Asojo et al., 2005). Because of its immunogenic and protective properties, *Na*-ASP-2 is being explored as a vaccine candidate against human hookworm disease (Bethony et al., 2005; Loukas et al., 2006; Mendez et al., 2008; Xiao et al., 2008). In adult *A. caninum*, at least four other ASPs (named *Ac*-ASP-3, *Ac*-ASP-4, *Ac*-ASP-5 and *Ac*-ASP-6) have been identified to date (Zhan et al., 2003). Another ASP-like molecule, designated neutrophil inhibitory factor (NIF), has been isolated and shown to play an immunomodulatory role by blocking the adhesion of activated neutrophils to vascular endothelial cells and the subsequent release of H_2O_2 from activated neutrophils (Moyle et al., 1994). NIF further interferes with the function of integrin receptors located on the cell surface, resulting in the inhibition of the aggregation and adhesion of platelets (Del Valle et al., 2003).

Clearly, investigating the structure of ASPs has major implications for understanding the molecular interactions between hookworms and their mammalian hosts. The topology of proteins of this group comprises an N-terminal PR (or CAP) domain, a hinge region and a variable C-terminal extension domain, such as the ion channel regulator domain in CRISPs, C-type lectin domain or the LCCL (=Limulus clotting factor C, Coch-5b2, and Lgl1) domain (Gibbs et al., 2008). This latter C-terminal domain is found in ASPs of mammals but is absent from invertebrates (Gibbs et al., 2008). To date, six SCP/TAPS proteins (see Table S1) have been characterized at the structural level, revealing the common fold of the core domain, first described for the PR-1 protein P14a from plants (Fernández et al., 1997). The SCP/TAPS core consists of an α-β-α sandwich in which the three-stranded anti-parallel β-sheet is flanked by three helices on one side, and a fourth helix on the other. These three, stacked layers are held together at the interfaces by a hydrophobic core. With the exception of GAPR-1 (Gibbs et al., 2008), the fold of the core domain includes three conserved disulphide bonds. In spite of this information, the structural classification of ASPs is unknown. Here, we establish, for the first time, that there are at least three distinct groups based on structural characteristics. Using this classification system, we determine the three-dimensional crystal structure of an entirely novel ASP (designated *Ac*-ASP-7; belonging to "group 2") that is highly up-regulated upon transition of the *A. caninum* L3 stage to parasitism, and examine its structure–function relationships by comparison with the known structure of *Na*-ASP-2 (Asojo et al., 2005) representing "group 1". In addition, we identify a metal binding site in the C-terminal extension of *Ac*-ASP-7 which might constitute a conserved feature of ASPs representing group 2.

2. Structure determination

Recombinant *Ac*-ASP-7 was produced as a secreted protein in the yeast *Pichia pastoris* as described for *Na*-ASP-2 (cf. Goud et al., 2005). Briefly, the open reading frame (ORF) of the mature protein (excluding the endogenous signal peptide, beginning at Leu-19) was cloned into pPIC-Zα, in frame with the cleavable N-terminal alpha-mating factor signal peptide encoded by the plasmid and C-terminal hexa-His tag. The secreted protein was purified from culture medium using nickel-NTA affinity chromatography and an AKTA UPC protein purification workstation (GE Health Care). Initial crystallization screens were carried out at 16 °C in sitting drops in 96-well MRC plates (Molecular Dimensions Ltd, Suffolk, UK) and our large in-house factorial collection (>1000 pre-formulated conditions). An ideal concentration of 4.7 mg/ml was determined. First crystals were obtained using either of the following conditions: 10% PEG2000, 20 mM KH_2PO_4, pH 8.0 or 20% MPD, 0.1 M TRIS, pH 6.8. The conditions were then refined to 15–30% MPD, and 0.1 M bis–TRIS, pH 6.5 or 0.1 M TRIS pH 7.0 or 7.5 in hanging drops using 24-well plates. Crystals appeared after 10 days. The X-ray crystal structure of *Ac*-ASP-7 was determined using multiple isomorphous replacement with anomalous diffraction (MIRAS) using data from two derivatives (ethyl mercury phosphate, $LaCl_3$) and one native crystal. Data sets were indexed with MOSFLM (Leslie, 1992), and scaling, truncation and analysis were performed using programs from the CCP4 suite (Collaborative Computational Project Number 4, 1994). The program autoSHARP was used to find heavy atom sites and conduct heavy atom refinement, density modification and solvent flattening. Data collection and phasing statistics are given in Table S2. Multiple rounds of manual model building with O (Jones et al., 1991) and Coot (Emsley and Cowton, 2004) were interspersed with computational refinement (rigid body refinement, positional and B-factor refinement) with moderate NCS restraints using Phenix (Adams et al., 2010). Structure refinement statistics are given in Table 1. The atomic coordinates and structure factors have been deposited with the Protein Data Bank (accession numbers 3s6s, 3s6u and 3s6v). Interface analysis was conducted using the PISA web server