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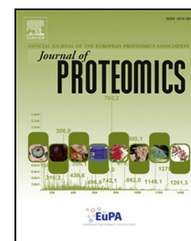

In vivo intravascular biotinylation of *Schistosoma bovis* adult worms and proteomic analysis of tegumental surface proteins

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A B S T R A C T

Schistosoma bovis is a blood-dwelling fluke of ruminants that lives for years inside the vasculature of their hosts. The parasite tegument covers the surface of the worms and plays a key role in the host–parasite relationship. The parasite molecules expressed at the tegument surface are potential targets for immune or drug intervention. The purpose of this work was the identification of the proteins expressed *in vivo* on the surface of the tegument of *S. bovis* adult worms. To accomplish this we used a method based on *in vivo* vascular perfusion of mice infected with *S. bovis* which allowed the labelling of the surface of the worms inside the blood vasculature. The biotinylation of parasite inside blood vessels prevents the handling of worms *in vitro* and hence possible damage to the tegument that could produce results that would be difficult to interpret. Trypsin digestion of biotinylated proteins and subsequent liquid chromatography and tandem mass spectrometry analysis (LC–MS/MS) resulted in the identification on the *S. bovis* tegument of 80 parasite proteins and 28 host proteins. The proteins identified were compared with the findings from other proteomic studies of the schistosome surface. The experimental approach used in this work is a reliable method for selective investigation of the surface of the worms and provides valuable information about the exposed protein repertoire of the tegument of *S. bovis* in the environmental conditions that the parasite faces inside the blood vessels.

Biological significance

To identify the proteins expressed on the surface of the tegument of *S. bovis* adult worms we used a method based on *in vivo* vascular perfusion, with biotin, of mice infected with *S. bovis* which allowed the labelling of the surface of the worms inside the blood vasculature. This methodology prevents the handling of worms *in vitro* and hence possible damage to the tegument that could produce results that would be difficult to interpret. This work is the first in which vascular perfusion has been used to investigate, *in vivo*, the protein exposed by an intravascular pathogen on its surface to the host, and provides valuable information about the exposed protein repertoire of the tegument of *S. bovis* in the environmental conditions that the parasite faces inside the blood vessels.

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1. Introduction

Schistosomiasis is a severe parasitic disease caused by flukes of the genus *Schistosoma* that affects humans and animals in many tropical and subtropical areas of the world. *Schistosoma bovis* is a blood-dwelling fluke of ruminants that, like human schistosomes, penetrates the host body through the skin until reaching a skin capillary vessel. It then migrates through the blood stream towards the lungs, where it remains for several days as a schistosomulum larva, after which it travels to the portal vein and mesenteric vessels, where it finally develops into the adult stage.

Schistosomes live for years inside the vasculature of their hosts, in contact with the vascular endothelium and the blood components involved in immune and haemostatic responses. Such a long survival time is achieved because schistosomes have developed diverse mechanisms to evade immune and haemostatic host responses, many of which are dependent on the properties of the parasite tegument [1–7].

The tegument of adult schistosomes covers the entire surface of the worms and is a unique outer-surface structure consisting of a cytoplasmic syncytium attached to underlying cell bodies by narrow cytoplasmic connections. The nuclei, ribosomes, endoplasmic reticulum, mitochondria and Golgi apparatus are located in these cell bodies, and their vesicular products – the so-called discoid bodies and multilaminate vesicles – are transported to the tegument syncytium *via* the connections. The apical surface of the tegument is made of normal plasma membrane overlaid by a membrane-like secretion, which has been termed as membranocalyx [8,9]. Together with the gastrodermis, this tegument constitutes a major interface between the parasite and its host, and it is critically involved in the complex host–parasite relationship (nutrient update, excretion, osmoregulation, *etc.*) [10]. Thus, the identification and characterization of schistosome tegumental molecules are essential for a better understanding of the host–parasite relationship and for defining novel immunological, pharmacological and diagnostic targets. Accordingly, many investigations carried out over the last five decades have focused on the identification and characterization of the schistosome molecules expressed on their tegument and surface membranes [9].

Major advances in the identification of proteins from the schistosome tegument have been made in the last decade as a consequence of the development of proteomics and the sequencing of the *Schistosoma mansoni* and *Schistosoma japonicum* genomes and transcriptomes (www.schistoDB.org) [11,12]. These studies have involved the study by proteomics of the whole or part of the tegument after fractionation with different techniques [9,13–20].

Since the accessibility of a protein in the tegument is an important issue when selecting target molecules for vaccines and drugs, studies aimed at identifying the proteins exposed on the worm surface are particularly interesting [15,17–19]. Two types of experimental approach have been used to achieve this: (i) The *in vitro* biotinylation of live worms with impermeant biotin reagents to label proteins with amine groups exposed either at lysine residues or at the N-terminus, followed by the purification of the biotinylated proteins and their analysis by mass spectrometry for identification [15,18]; (ii) Enzymatic shaving by incubation of intact worms with hydrolytic enzymes, trypsin or phosphatidyl-inositol specific phospholipase C (PiPLC) to release peptides from the surface-exposed proteins and peptide analysis by LC–MS/MS [17,19].

All these studies have illustrated the dynamic nature of the schistosomal tegument and have provided invaluable information about the repertoire of tegumental molecules that are expressed on the tegument surface and their relative locations at the plasma membrane and membranocalyx. In such studies, besides several proteins of host origin (immunoglobulins, complement factors and the CD44 and CD90 host cell surface proteins), it has also identified some schistosome transporters, enzymes and structural membrane proteins, and a variable range of cytosolic and cytoskeletal proteins, whose presence on the surface has been attributed by some authors to tegument damage and the subsequent release of parasite inner cytosolic or structural molecules, and which are interpreted as “false” identifications from the tegument surface [18,20,21]. It is known that one of the major limitations of *in vitro* work with schistosomes is the fragility of the worms. Thus, even though the worms are handled with great care it cannot be ruled out that some tegumental damage may lead to biotinylation or enzymatic digestion of internal proteins [20].

Regarding the tegument of *S. bovis*, up to date only three studies have attempted to analyse its composition [16,17,22]. In the first two studies, two dimensional (2D) proteomic maps of the tegument were constructed, and the more abundant proteins, the antigenic ones and the glycoproteins were identified by mass spectrometry analyses of the corresponding spots sliced from the 2D gels [16,22]. The third work, aimed at identifying the proteins expressed on the outermost layers of the tegument structure of adult worms, was the first to apply the enzymatic shaving technique in a schistosome species. Male and female worms were separately subjected to soft trypsin digestion and a wide range of tegumental proteins were identified – most of them for the first time in *S. bovis* – , and some differences were being observed in the tegumental protein repertoire of both sexes [17].

The aim of the present work was to perform an *in vivo* identification of the host-exposed tegumental proteins from *S. bovis* adult worms. To accomplish this, we used a method that permits the labelling of the surface of the worms inside the blood vasculature and that is based on the vascular perfusion of mice infected with *S. bovis* with a biotin ester reagent. In this way, the proteins of the tegument surface of the worm entering into contact with the perfusion fluids became biotinylated and were efficiently purified by streptavidin affinity chromatography. Trypsin digestion of the biotinylated proteins and subsequent liquid chromatography and tandem mass spectrometry analysis (LC–MS/MS) resulted in the identification on the *S. bovis* tegument of 80 parasite proteins and 28 host proteins.

2. Material and methods

2.1. Experimental animals and parasites

Thirty-five NMRI mice weighing 30–35 g (Harlan Laboratories Models, S.L.) were used. All mice were infected with 200

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