

The Proteome of hatched *Schistosoma mansoni* eggs: defining the unique composition of the subshell envelope

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Schistosomiasis, caused by digenetic flatworms of the genus *Schistosoma*, is the second most significant parasitic disease globally. The disease is prevalent in tropical and subtropical regions, particularly in impoverished communities lacking access to clean water and proper sanitation. Among the clinical manifestations, acute symptoms include dermatitis and Katayama fever. In chronic cases, granulomatous inflammatory responses are one of the main factors contributing to the severity of the disease. Granuloma formation is induced by antigens secreted from mature eggs lodged in the host's liver tissue. Egg secretion is believed to modulate the inflammatory response, leading to hepatic and intestinal granuloma formation. Studies on the various components of the parasite's eggs are scarce, and the host interaction mechanisms remain largely unknown. This study aims to identify the proteomic constituents of the *S. mansoni* egg subshell envelope, through a mass spectrometry-based shotgun approach. Livers from C57BL/6 mice were collected after 7 and 12 weeks of infection with *S. mansoni* cercariae. Liver homogenates were digested using bovine trypsin to release entrapped eggs. These were obtained by sequential sieving using 300 µm and 180 µm filter meshes. Then, the recovered eggs were hatched in mineral water. After removal of live miracidia, the hatched eggs containing the subshell envelope were manually collected with the aid of a stereomicroscope and subjected to enzymatic digestion using MS grade trypsin. The supernatant was analyzed using a Dionex UltiMate 3000 UHPLC platform coupled to mass spectrometry (Q Exactive/Thermo Scientific). Mass spectral data were interrogated against the *S. mansoni* proteome database using Peaks 8.5 Studio software. In total, 83 groups of proteins were identified. Among these, the classically described markers of the subshell envelope were identified, namely glycoprotein IPSE/alpha-1, kappa5 and omega 1, known for their potential role in immune modulation. In addition, polymorphic variants of mucins, BPTI/Kunitz-type inhibitors, allergens and hepatotoxic proteins, were also identified. Quantitative data revealed that the 20 most abundant proteins constitute 84.06% of the identified proteome. The identified molecules may perform a variety of functions ranging from egg migration to granuloma formation. The newly identified molecules may contribute to understanding the mechanisms by which the parasite regulates the host immune system resulting in egg translocation from tissues to intestinal lumen. Further studies are underway to ascertain the exact location of the identified constituents in the *S. mansoni* egg to allow for possible inferences on their role in this complex host-parasite interaction.

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