Parasitology International 66 (2017) 816–820

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Short communication

Differential distribution and biochemical characteristics of hydrolases among developmental stages of *Schistosoma mansoni* may offer new anti-parasite targets

Milagro Fernández-Delgado [a,1], Jackeline Cortez [a], Guiden Sulbarán [a,2], César Matos [a], Renzo Nino Incani [b,*], Diana E. Ballén [a], Italo M. Cesari [a]

[a] Centro de Microbiología y Biología Celular, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela
[b] Departamento de Parasitología, Facultad de Ciencias de la Salud, Universidad de Carabobo, Valencia, Venezuela

ARTICLE INFO

Article history:
Received 2 June 2016
Received in revised form 27 September 2016
Accepted 27 September 2016
Available online 28 September 2016

Keywords:
Schistosoma mansoni
Enzymatic activity
Phosphatases
Glycosidases
Aminopeptidases

ABSTRACT

Schistosoma mansoni enzymes play important roles in host-parasite interactions and are potential targets for immunological and/or pharmacological attack. The aim of this study was to comparatively assess the presence of hydrolytic activities (phosphatases, glycosidases, aminopeptidases) in soluble (SF) and membrane (MF) fractions from different *S. mansoni* developmental stages (schistosomula 0 and 3 h, juveniles, and adult worms of 28 and 45 days-old, respectively), by using simple enzyme-substrate microassays. Our results show and confirm the prominent presence of alkaline phosphatase (AlP) activity in the MF of all the above parasite stages, highlighting also the relevant presence of MF-associated α-mannosidase (α-MAN) activity in juveniles. A soluble AlP activity, together with β-N-D-acetylglucosaminidase (β-NAG), and α-MAN activities, was detected in SF of schistosomulum 0 h. Soluble β-NAG, α-MAN, acid phosphatase (AcP), leucin (LAP) and alanine (AAP) aminopeptidase activities were also seen in the SF of the other different developmental stages. This work shows different soluble and membrane-associated hydrolytic capacities in each *S. mansoni* developmental stage from schistosomula to adults that might be exploitable as potential new targets for immune and/or chemoprophylactic strategies.

Schistosomiasis is a neglected tropical disease affecting 200 million people in 76 countries. It is caused by flukes of *Schistosoma* genus, which have complex life cycles in definitive human and intermediate snail hosts. *Schistosoma mansoni* arises across sub-Saharan Africa, Middle East, Brazil, Venezuela and West Indian islands. Infection occurs when the free-swimming larvae or cercariae penetrate the host skin, transform into schistosomula, travel by the blood vessels to the lungs, migrate to the hepatic portal vein where they grow to juveniles, and then to the mesenteric vessels, where they sexually mature into adults, copulate and acquire nutrients from blood to support the production of eggs.

There are still high morbidity and mortality rates by *S. mansoni* worldwide and an urgent need for new therapeutic approaches [1,2]. Current research focuses to control schistosomiasis by the identification of new parasite targets, drugs and vaccine development [1,2]. Recently, the *S. mansoni* genome sequence was reported [3] and several transcriptional and proteomic studies have identified new possible parasite target molecules [1,4–7]. In this context, *S. mansoni* enzymes have been shown to play key roles in the host-parasite interactions and are usually considered potential targets for immunological and/or pharmacological attack [1,4,6–10]. Many enzymatic changes occur during the intra-host parasite development and are mostly related to parasite adaptation to successive different host environments during penetration, migration, growth and reproduction; for instance, cysteine and aspartic proteinases may play a key survival role for *S. mansoni* during host penetration [10]; hemoglobinases and cathepsin B are involved in hemoglobin degradation that feeds adult parasites [11]; through its immune modulating properties, cathepsin B may be also involved on the effectiveness of the immune response against adult worms [12], which might have important implications in the development of immunoresistance to schistosomiasis [9,12,13]; aminopeptidases associated with the integument of the parasite may be involved in parasite's growth within the mammalian host [6–7,13], besides their possible role in the metabolism of organic phosphate substrates [14]; alkaline phosphatase generates the immunosuppressant adenosine which benefits adults by attenuating the potential for inflammation and creating a less immunologically hostile, local environment for schistosomes [15]; a highly expressed enolase

* Corresponding author.
E-mail address: rincani@uc.edu.ve (R.N. Incani).
[1] Present address:
Centro de Ingeniería de Materiales y Nanotecnología, IVIC, Venezuela.
[2] Present address:
Centro de Biología Estructural- Humberto Fernández-Morán, IVIC, Venezuela.

http://dx.doi.org/10.1016/j.parint.2016.09.015
1383-5769/

in schistosomula, as well as adult worms and eggs, is able to bind to and enhance the activation of human plasminogen to control hemostasis around the worms [16]. Recently, it has been also suggested that a protein kinase mediates cercarial adaptive responses to host skin molecules including fatty acids and the physiological response of 24 h somules during skin invasion and migration [17].

In the present work, several hydrolases were studied in soluble (SFs) and membrane (MFs) fractions from mammalian stages of *S. mansoni* to identify differences in enzyme distribution and capabilities that could be exploitable as targets for innovative anti-parasite strategies.

The life cycle of *S. mansoni* (Venezuelan JL strain) was maintained at IVIC by passage through hamster and the intermediate snail host *Biomphalaria glabrata* (Venezuelan Guacara strain). Animal work was conducted in accordance with international standards adopted by the Committee of Bioethics for Animals (COBIANIM) at IVIC (reference N°1450). Different developmental stages of *S. mansoni* were collected. Schistosomula 0 h-old (Sm0h) were obtained after mechanical transformation of freshly-released cercariae from infected snails [18]. Sm0h was selected because is close to free-living cercaria, a stage that makes the first contact with the vertebrate host, just before penetration and adaptation to the host; Sm3h stage was selected because, as compared to Sm0h, it could be representative of an initial host tissue stage where we could possibly identify some of the initial changes in parasite enzyme expression needed for adaptation to host; *ex vivo* Sm28d was selected as an already well adapted stage to the host environment, being pre-adult young worms, and should presented differences in comparison to Sm3h and the adult stage; Sm45d was selected because it is the adult and sexually differentiated mature worm stage. A number of Sm0h was incubated for 3 h at 37 °C in Minimum Essential Medium to obtain 3 h-old schistosomula (Sm3h). Juveniles and male and female adults were collected after portal perfusion 28 and 45 days post-infection, respectively. Sm0h (~68,375 parasites/mL), Sm3h (~152,900 parasites/mL), Sm28d (~80,000 parasites/mL), and Sm45d (500–1000 parasites/mL) were homogenized separately in 50 mM Tris/HCl pH 8.0 containing 1 mM $MgCl_2$ (Tris-Mg buffer). Whole parasite homogenates were ultracentrifuged at 100,000 ×*g* for 2 h at 4 °C. Supernatants of each parasite stage were considered as SFs. Membranous pellets were washed twice with Tris-Mg buffer, resuspended and ultracentrifuged as above; washed pellets were resuspended in the same buffer, mixed v/v at room temperature with water-saturated *n*-butanol, and strongly agitated in vortex. Suspensions were centrifuged for 15 min at 14,000 ×*g* at 4 °C; the upper organic phase was discarded and the lower aqueous phase extract (containing solubilized *n*-butanol-resistant membrane proteins, glycoproteins, and diverse protein antigens [19] was recovered, dialyzed overnight against Tris-Mg buffer and considered as MF. Protein concentration in SFs and

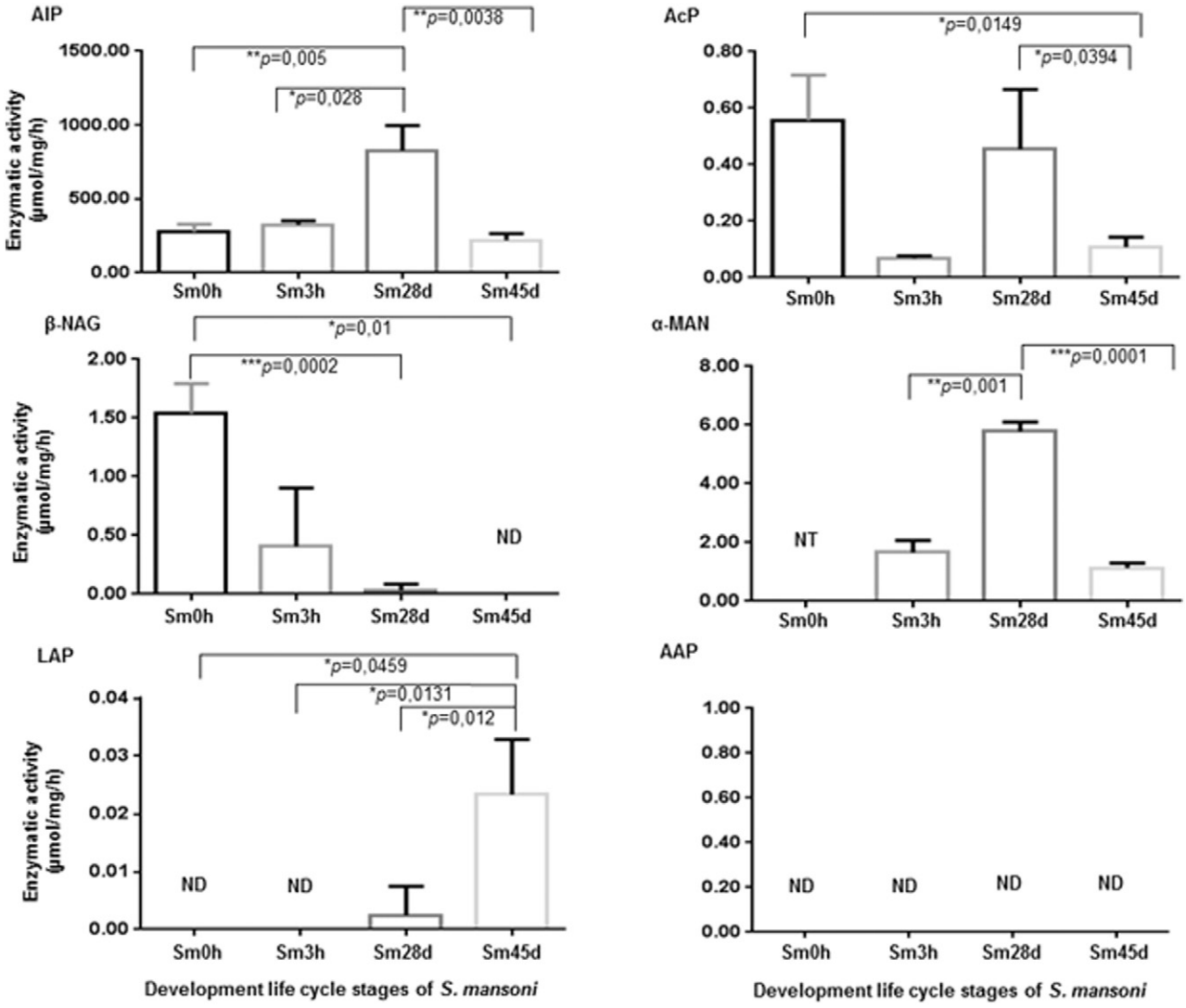

Fig. 1. Activities of phosphatases AlP and AcP, glycosidases β-NAG and α-MAN, and aminopeptidases LAP and AAP in MFs from different developmental stages of *S. mansoni*. ND: not detected; NT: not tested.