RESEARCH NOTE

Lactate Dehydrogenase: Sequence and Analysis of its Expression during the Life Cycle of *Schistosoma mansoni*

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Key words: *Schistosoma mansoni* - lactate dehydrogenase - expression pattern

Schistosoma mansoni is one of the causative agents of schistosomiasis, a parasite disease affecting 200 million people. Schistosomes are the only trematodes which are sexually dimorphic. During their development, the parasite undergo profound morphological and biochemical changes. Free-living cercarial emerge from their invertebrate snail host into fresh water using oxidative glucose metabolism to provide high energy levels in their efforts to find and penetrate into a final vertebrate host (BEP Van Oordt et al. 1989 *Parasitology 98*: 409-415). During the host invasion cercariae loses their tails and transforms into schistosomula. Biochemical studies suggest that this transformation is accompained by a transition from an aerobic to a more anaerobic energy metabolism (WN Von Kruger et al. 1978 *Comp Biochem Physiol 60-B*: 41-46, DP Thompson et al. 1984 *Mol Biochem Parasitol 13*: 39-51, Van Oordt et al. 1989 *loc. cit.*). In the vertebrate blood stream the schistosomula develop into adult worms which generate large amounts of lactate using an anaerobic metabolism (GC Coles et al. 1972 *Nature 240*: 488-489, EL Schiller et al. 1975 *J Parasitol 61*: 385-389, BEP Van Oordt et al. 1985 *Mol Biochem Parasitol 16*: 117-126).

The pathway glycolytic enzymes of *S. mansoni* have been used to study new strategies for the prevention or treatment of schistosomiasis. Such strategies have used the hexokinase (C Shoemaker et al. 1995 *Exp Parasitol 80:* 36-45), STPI (C Shoemaker et al. 1992 *Proc Nac Acd Sci USA 89*: 1842-1846) and SGAPDH (V Goudot- Crozel et al. 1989 *J Exp Med 170:* 2065-80). Another enzyme used in this strategy is the lactate dehydrogenase (LDH) which catalyzes the interconversion of L- Lactato and piruvate with nicotinamide adenine dinucleotide (NAD^+) as coenzyme. The LDH enzyme is widely distributed among animals, plants and bacteria. In mammals and birds the LDH-A, LDH-B and LDH-C subunits are encoded by Ldh-a, Ldh-b and Ldh-c genes, respectively. The three homotetrameric isozymes LDH-A_4, LDH- B_4, LDH- C_4 possess distinct physical, catalytic and immunological properties (RS Holmes 1972 *FEBS Letters 28*: 51-55, CL Markert et al. 1975 *Science 189*: 102-114).

We report here the sequencing and characterization of *S. mansoni* LDH gene. A cDNA, clone SMJ103 encoding a putative LDH (Sm LDH), isolated from a directional cDNA library constructed with mRNA of *S. mansoni* adult worms NMRI strain (GR Franco et al. 1995 *Gene 152*: 141-47). This cDNA clone containing a Not I restriction site at 3'end and a Hind III site at 5'end was subcloned in pUC19 and sequenced using a fluorescent M13 and reverse primers in a automated sequencer (ALF-Maneger, Pharmacia).

The complete sequence of SmLDH shows at the 3'end a polyadenylation signal with the consensus sequence AATAAA. The deduced translation product of 333 amino-acids has a molecular mass of 34000 Da. The deduced amino-acid sequence was examined for homology with other LDH family proteins. The aminoacid sequence exhibits 65% identity with other LDH enzymes. The majority of the residues responsible for the substrate, coenzyme binding and catalysis sites are well conserved in *S. mansoni* LDH like others LDH of various organisms. However, several notable differences in aminoacids composition were observed in SmLDH that contained several distinctive single aminoacid insertions and deletions compared to other LDH enzymes.

To estimate the relative transcript levels of this enzyme, total RNA from eggs, miracidium, cercariae, schistosomulum male and female adult worms was obtained using Trisol reagent (Gibco). About 5 mg the total RNA from each developmental fase was spotted onto nylon membrane

This work was supported by grants from Fapesp, CNPq and Finep.
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Received 4 May 1998
Accepted 31 August 1998

(Hybond^{TM} - N+) and crosslinked by ultraviolet radiation. The RNA Dot Blots were hybridized with a cDNA of SmLDH labelled with (a P^{32}). Surprisingly, transcripts encoding Sm LDH are expressed in larval schistosomes with higher levels than in adult worms.

LDH isoenzymes were studied by eletrophoresis in 12% horizontal starch gels of potato amide (Sigma). Briefly, fresh adult worms (LE strain) were washed out of blood and debris by rinsing three times in ice-cold PBS solution. Female and male worms were placed in a glass tube in 100 ml of distilled water, and the parasites crushed with a ground glass stopper at room temperature. The resulting homogenate was immediately absorbed on 6x5mm of Whatman no.1 filter paper. Electrophoresis was carried out for 5 hr at 4°C, and LDH (E.C.1.1.1.27) activity was reveled by incubating each gel slice with the corresponding staining solution in the dark at 37°C (M Fletcher et al. 1981 *Exp Parasitol 52*: 406-421). This technique showed sexual differences in mobility and number of LDH bands as described by GC Coles et al. (1970 *Comp Biochem Physiol 33:* 549-558) and M Fletcher et al. (1981 *Am J Trop Med Hyg 30:* 406-421) to different geographic populations. The difference in migration and number of isoenzymes may be associated to a putative preferential expression of the gene in male worm suggesting a possible alteration in carbohydrate metabolism or energy production in adult worms. Obviously this hypothesis requires further investigation.