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Histochemical localization of Malate Dehydrogenase activity in *Schistosoma spindale*

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ABSTRACT

Malate Dehydrogenase is one of the key enzymes of the tricarboxylic acid cycle. It oxidizes l-Malate into oxalo-acetate. It is found in all eukaryotic cells. It has been found that Malate dehydrogenase plays an important role in the metabolism of the trematodes, The significance of the MDH in the active sustenance of the trematode cannot be denied. As the parasite, *Schistosoma spindale* resides in the blood of the host, *Bubabus bubalis* an anaerobic mechanism is found to be operative. MDH is found to have an alternative pathway adapted by the parasite in response to the parasitic adaptations. *S.spindale* extracts the nutrition available in the blood to lead a parasitic life. The suckers help the parasite in nutrition as well as movement. The present investigation, an attempt to histochemically localize malate dehydrogenase enzyme in the sections of S.*spindale.*

1. Introduction

The TCA cycle is of immense importance to parasitic helminths as its energy yield is much higher that that of the Embden-Meyerhof pathway. Malate Dehydrogenase, a TCA cycle enzyme is a large protein molecule with subunits weighing between 30 and 35 kDa.(1). Inside the cell, MDH occurs in two forms as the mitochondrial (M-MDH) and soluble or cytoplasmic (S-MDH) according to its location (2). It is the mitochondrial form of MDH that forms the component of the TCA cycle. It is of great significance in the metabolism of carbohydrates in parasitic helminths. It leads to the formation of succinate via fumarate involving a higher yield of energy (3). To demonstrate the enzymatic study like Malate dehydrogenase in the tissues of helminths histochemistry is the best proven option. It involves techniques that provide biochemical and molecular information about the structure and function of cells, tissues and organs. Histochemical work tends to display the enzymatic reactions very clearly as it deals with the identification of chemical components in cells and tissues.. The present research work have been undertaken to localize histochemically MDH activity in the trematode, *Schistosoma spindale*.

2. Materials and Methods

Histoenzymological Studies (Malate Dehydrogenase)

Method: Histoenzymological studies involves the localization of enzymes in parasite by using a cold neutral fixative and sectioning the tissue blocks on a cryostat or freeze microtome. 10% neutral buffered formalin was used to fix the cryostat sections 12-16 µ thick. These were put in incubating medium for 30 minutes to 1 hour at 37^{o}C. The sections were immersed in 10% formal saline for 10-15 minutes. Sections were then washed in tap water for 2 minutes. Distilled water was used to rinse the sections and mounted in glycerin jelly. Blue formazon deposits(NBT) were formed at the sites of enzyme activity[4].

3. Results

The Malate Dehydrogenase activity was localized as bluish diformazon granules in the parasite.

a. The tegument of *S.spindale* showed notable activity of malate dehydrogenase(Fig. 1,2,3,4,5,)
b. The muscular layer of the body wall and the parenchyma showed showed intense activity of MDH.(Fig 3,4).
c. Remarkable activity of MDH was seen in the gynaecophoric canal. (Fig. 8,9,10)
d. Significant activity of MDH was seen in the male and the female *S.spindale.*(Fig.6,8,9,7)
e. Suckers showed profuse MDH activity. (Fig.10,11). A thick bluish colouration of diformazon deposits was observed in the ventral sucker and oral sucker(Fig.10,11)

4. Discussion

The musculature constituting longitudinal muscular layer, circular muscular layer, subcuticular cells shows significant activity. It can be accounted by the fact that there is rapid energy catabolism for release of ATP in the contracting muscles of the body wall as the parasite is in continuous movement in the blood. Besides intense enzyme activity was also found in the muscles of the gynaecophoric canal. The muscles of the gynaecophoric canal are activitly involved in accommodating the female and more energy requirement can be generalized. operation of Kreb's cycle partially or fully, can be substantiated. The suckers of the parasite are among the privileged tissues in having high level of MDH as the suckers are the regions always in action engulfing the contents of blood for nutrition and adhering to the blood vessels. MDH activity of helminth have been reported from various parasites like *Hymenolepis diminuta*, Moon et al.(1977); *Ascaris suum,* Bueding and Saz(1968), in cercariae and adult *S.mansoni*, Coles(1973) , in *S.japonicum*, Huang(1980), in *Tritrichomonas foetus,* Hrdy and Mertens(1993), in *Echinococcus granulossus,* Aguero et

al.(2004), in *Brugia malayi*, Bhadary et al.(2006)[5-11]. These works corroborate and confirm the importance of the role played by malate dehydrogenase in the metabolism of the parasite. The work undertaken by the investigators, validates the role of MDH in the suitable adaptation of parasitic life of *S.spindale*.

5. Conclusion

Helminths comprise a diverse parasitic and free-living worms with a long evolutionary and historical background. They have adopted the parasitic lifestyle and have become exquisitely well adapted over time to the immune system of the host. Schistosomes are highly pathogenic causing visceral inflammation and liver fibrosis to humans and animals. It is time to introspect the enzyme activity governing the adaptation of the parasite to lead a life in ecosystem of blood, its components in the tiny tunnel of blood vessel. However, the metabolic pathway in helminths do not involve a classical tricarboxylic cycle. Von brand(1973) pointed out that helminth metabolism frequently differed from the classical pattern found in higher animals and was similar in many respects to that found in anaerobic microorganisms[12]. Rogers(1976) observed that the adult trematode, *Schistosomatium douthitti* possessed some of the Kreb's cycle enzymes which were reported to be completely absent in the miracidium[13]. These findings emphasize that the environmental factors play a pivotal role in the metabolic pathways adapted by the parasites. In an anaerobic environment where the oxygen content is very low, the parasites may exhibit a variation in the kreb's cycle enzymes ranging from very low to nil, an adaptation correlated with their microniche. The present work is an added dimension to the unseen parameters of research of malate dehydrogenase in *S.spindale*.

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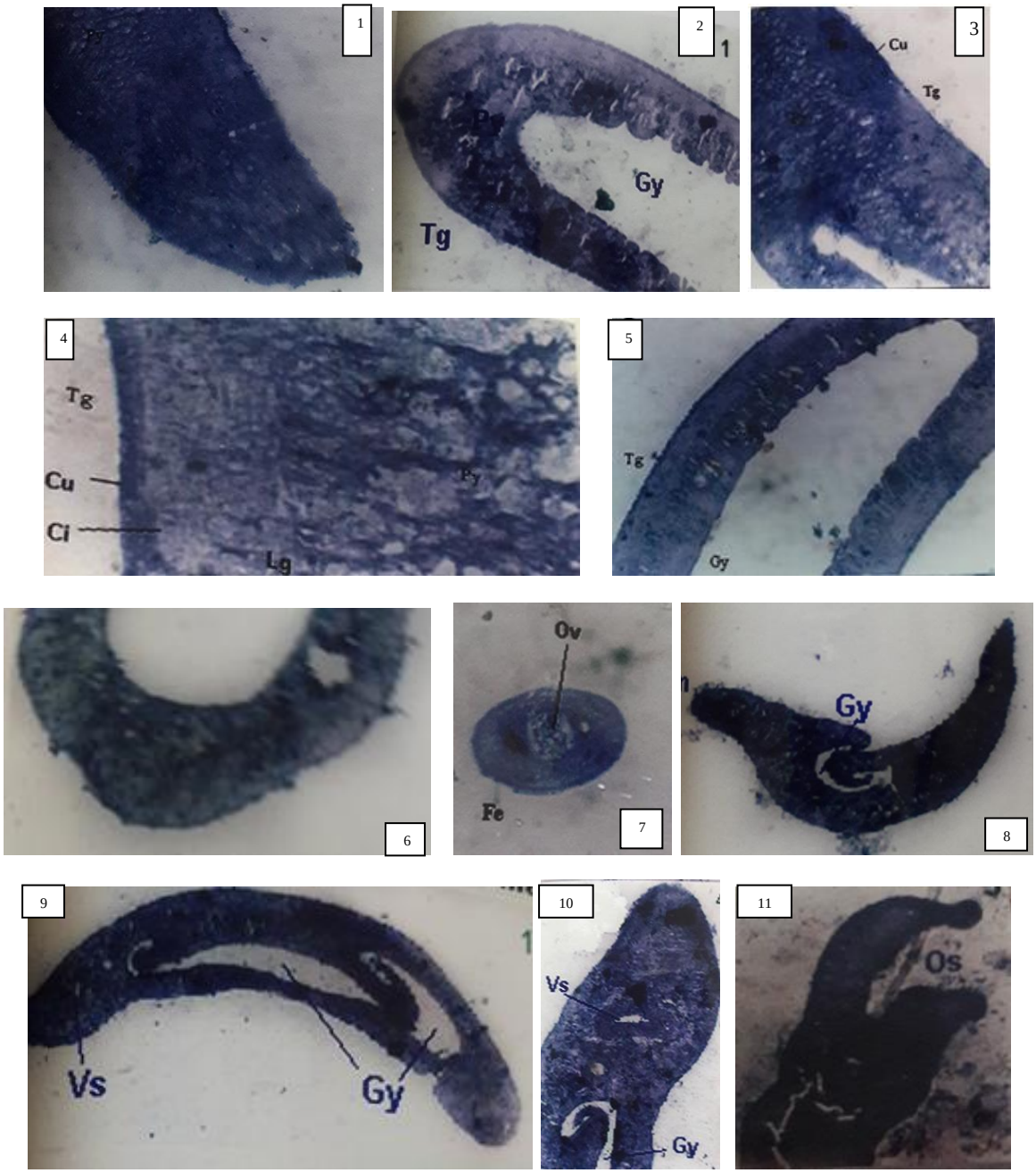

Fig.1 , 2.,3,4,5. MDH activity in body wall of *S.spindale*. Fig 4. Magnified view of the Section of the male *S.spindale* showing MDH activity in the body wall and parenchyma. Fig.7. MDH activity in the transverse section of the female *S.spindale*. Fig.6,8,9. MDH activity in section of male *S.spindale*. S. Fig. 8,9,10. MDH activity in the gynaecophoric canal of male *S.spindale*. Fig. 10. MDH activity in Ventral sucker of the male *S.spindale*. Fig 11. MDH activity in the oral sucker of male S.*spindale*.

(Labellings are M-male, Fe-Female, Cu-cuticle, Tg-Tegument, Ms-Musculature,Ci-Circular layer of Musculature, Gy-Gynaecophoric canal, Vs-Ventral sucker, Os-Oral sucker, py-parenchyma,Ov-Ovum)

