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Esterase Activity in Some Digenetic Trematodes

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Introduction

Histochemical demonstration of the esterase activity has been carried out in a number of parasitic flatworms by many workers but very little is known about the isoenzyme patterns of esterases in helminth parasites apart from a few reports on *Fasciola hepatica, Schisotosoma* spp, and *Ascaris lumbricoides* (see von Brand, 1973).

The present communication deals with the histochemical localization as well as isoenzyme patterns of esterase obtained by polyacrylamide disc gel electrophoresis, in some important and hitherto unstudied trematode parasites. They include *Isoparorchis hypselobagri* from the swim bladder of the catfish *Wallago attu, Gigantocotyle explanatum* from the liver and *Gastrothylax crumenifer* from the rumen of the water buffalo, *Bubalus bubalis.*

Materials and Methods

Details of the collection of specimens have been given elsewhere (Siddiqi *et al.*, 1975).

Histochemical study

The worms were fixed in 5% formalin, buffered to pH 7 with 0.2 M phosphate, for 24 h at 4°C. They were then rinsed thrice for 5 min in cold (4°C) distilled water. The fixed worms were sliced at 7 to 10 μm on a Reichert Wein 'OmP' freezing microtome. Frozen sections were directly transferred to Petri dishes containing the test medium. Esterase activity was demonstrated by the standard technique described by Pearse (1960), using 5-bromoindoxyl acetate (5-BIA) as the substrate. Control Sections were incubated in the test medium from which the substrate was omitted. The effect of eserine sulfate, a cholinesterase inhibitor, was seen by first incubating sections for 1 h in buffered solution of 10 μM of the inhibitor followed by incubation in the standard test medium containing the inhibitor at the same molarity.

Polyacrylamide disc gel electrophoresis

The crude enzyme preparation was obtained by homogenizing the parasites in a Potter-Elvehjem glass-teflon tissue grinder using 1 g fresh tissue per 4 ml distilled water. During grinding the homogenizer was kept in an ice-bath. The homogenates were frozen, thawed and centrifuged at 12,000 g in a high speed centrifuge (Unipan Type 310 Poland) for 30 min at 4°C. In some cases, tissue extract was obtained by repeated freezing and thawing of the whole parasite. The protein concentration was determined by the method of Lowry *et al.* (1951).

Polyacrylamide disc gel electrophoresis

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was carried out according to Davis (1964). The gels contained 10% acrylamide and 0.18% N, N′-methylenebisacrylamide. The gels were loaded with 50 μl aliquots containing 50–85 μg protein. Electrophoresis was conducted at 4°C in a Tris-glycine buffer system (pH 8.9) and a current of 4 mA/gel column (6×75 mm) was maintained until the tracking dye (bromophenol blue) had moved about 40 mm in the small pore gel. This usually takes about 2 h. Following electrophoresis, the gels were immediately removed from the glass tubes and the esterases were demonstrated according to the method of Hunter and Burstone (1960). The gels were incubated for 3 h at 37°C in a test medium that contained 10 mg 5-BIA in 2 ml of ethoxyethanol, 15 ml 0.2 M Tris buffer of pH 7, 5 ml 0.05 M potassium ferrocyanide, 5 ml 0.05 M potassium ferricyanide, and 15 ml distilled water. For controls the gels were incubated in the standard test medium without the substrate.

The gels with their cathodal end at the top, were photographed against a black background by employing a special light arrangement in the form of a narrow beam which was focussed on the top of the gel thereby rendering the bands visible for photography. The isoenzymes were consecutively numbered in order of their anodic mobility with the most anodal isoenzyme designated as E1.

Results

Histochemical study

The distribution of esterases in the various parts of the parasite body is shown in microphotographs (Figs. 1, 2).

Tegument: High concentration of esterases are seen throughout the tegument with species differences in the intensity of the reaction. Strong enzyme activity is seen in the tegument of *G. crumenifer* and *I. hypselobagri*, whereas, the enzyme activity is moderate in the tegument of *G. explanatum* (Fig. 1; A,B,C).

Suckers: Strong esterase activity is seen in the tegumental lining of both the oral and ventral suckers, as well as in the gland cells. Radial muscles in the sucker, however, show poor esterase activity (Fig. 2A).

Digestive system: Strong activity is found throughout the gastrodermis in all the species studied. The reaction product is particularly localized in the musculature of the intestine and there is no indication of the enzyme activity in the lumen of the gut. Moderate reaction is also seen in the pharyngeal muscles (Figs. 1A, 2B).

Reproductive system: The reaction product in the form of dense granules are found to be irregularly distributed in the testes. The cirrus and the associated structures are also strongly positive for the enzyme activity (Fig. 2B). In the female reproductive system, intense activity is seen in the ovaries, uterus, the area surrounding the genital pore, ootype and the associated structures (Fig. 1A, B,C).

Nervous system: The esterase activity is present throughout the nervous system in all the species studied. Intense activity is seen in the nerve fibers which arise from the cerebral ganglia and form a network throughout the parenchyma and the tegument. A very dense reaction is seen in the cerebral ganglia which lie immediately posterior to the pharynx and are connected by the broad anterior commissure. The main anterior nerve cords are also strongly stained for esterase (Fig. 1D).

Inhibitor study: As a result of the treatment with eserine sulfate the enzyme activity in the nervous system is inhibited, whereas, degree of inhibition of the enzyme activity in the tegument, the suckers, the testes and ovaries, the gastrodermis and the cirrus is comparatively less.

Electrophoretic study: The polyacrylamide gel electropherograms of isoenzyme patterns of esterases are shown in Fig. 3.

Five zones of esterase activity are resolved in the extract of *I. hypselobagri* (Fig. 3A). Esterases E1 and E5 show the most intense staining reaction and migrate as compact bands. Esterases E2, E3 and E4 are of low intensities and migrate as streaks. Esterases E1, E2 and E3 are anodal isoenzymes, whereas, E4 and E5 are cathodal fractions.

In *G. explanatum* the esterases are resolved into four isoenzymes (Fig. 3B). Esterases E1 and E2 are of low intensities and appear as streaks. E3 and E4 are the major fractions where the former appears as a compact band. The anodal isoenzymes are represented by E1 and E2 and the cathodal by E3 and E4. Esterases in *G. crumenifer* are resolved into at least three fractions (Fig. 3C). The middle zone bands, E1 and E2 are very faint and appear as streaks. The cathodal fraction E3 is a compact band showing strong enzyme concentration.

Discussion

The presence of esterase activity in the tegument of the trematodes investigated is in agreement with those reported in *F. hepatica* (Barry *et al.*, 1968), in many species of schistosomes (Fripp, 1966) and in the tegumental and subtegumental cells of the daughter sporocyst of *Cercaria bucephalopsis haimaena* (James and Bower, 1967). It has been suggested that esterases have a role in the transport of nutrients and excretory products across the cell membrane and in the breakdown of the incoming nutrients. In *F. hepatica* the acetylcholinesterase activity in the tegument was found in those sites which constitute the host-parasite interface, that is the ventral tegument and the tegumental lining of the oral and ventral suckers (Halton, 1967b). A role of the enzyme in the passage of substances across the membrane was suggested.

The distribution of esterase in the gastrodermis is similar to those previously described by Halton (1967a,b), Davis *et al.* (1969), Mandawat and Sharma (1978) in a number of digenetic trematodes.

All the three parasites investigated exhibit strong esterase activity in their suckers. The enzyme was found concentrated all along the tegumental lining of the suckers in the form of well demarcated area which appeared to be associated with the secretory activity. Ohman (1966) observed that in strigeid trematodes, the esterases of different kinds were secreted to the exterior by the lappets and gland cells in the adhesive organ. These secretions were believed to be lipolytic and supposed to attack the plasma membrane, resulting in the breakdown of the host tissue at the point of attachment.

The distribution of esterase in the tegument, gut and attachment organs of the species studied, corresponds to the distribution of phosphatases in these regions (Authors in press). Although the functions of esterases are not known with certainty, it appears most likely that like phosphatases, the esterases are also involved in the digestive process in trematodes. They may bring about lysis of the host tissue at the host-parasite interface. This assumption supports the view of Lee (1962 a) and Halton (1967 b) that the esterases are involved in the extracellular digestion.

Moreover, it is noteworthy that in this investigation the esterase activity is strongest in the tegument of *G. crumenifer*, a parasite of the gastrointestinal tract. In seeking an explanation to this we assume that the parasite lies bathed in a habitat where there is plenty of semidigested food. As a result, the extracorporeal digestion in this parasite appears to be a primary choice and most of the process of food intake might have been carried out by the tegument and hence there is an accumulation of larger quantity of esterase.

In the reproductive system the testes, ovaries and vitellaria were found strongly

positive for esterase activity. This is in agreement with the similar findings reported by Halton (1967 b) for *F. hepatica*, Barry *et al.* (1968) for the basement layer of the testes in *F. hepatica*, Sood (1977) for *Glossimetra orientalis*, and Mandawat and Sharma (1978) for *P. cervi*. While the esterase activity was uniformly distributed in the ovary and the vitellaria, the reaction product in the testes was found scattered in the form of dense granules. These granules are supposed to be associated with lipid droplets. According to Halton (1967 b) the esterases in the reproductive organs may function in the hydrolysis of lipid and other metabolic processes, and may facilitate the supply of the nutrients to the developing germ cells. In *A. lumbricoides* esterases have been found associated with mitochondria and the catabolism of lipid (Lee, 1962 b.) In other reproductive structures such as uterus, cirrus and oviduct the enzyme may be associated with the neuromuscular activities.

In the entire nervous system the reaction is inhibited by eserine, whereas, in all other organs there is a reduction in the enzyme activity but a certain percentage still remains demonstrable. The inhibition of esterase activity in the nervous system indicates that the predominant esterase present in the nervous system is cholinesterase since eserine sulfate is a specific inhibitor of the latter. The suppression of reaction in other organs, after the treatment with this inhibitor shows that besides nonspecific esterases there also exists cholinesterase in those organs of the parasite body.

Acetylcholinesterase has been reported in many parasitic helminths and is supposed to play some role in neurotransmission, and is also known to act as a biochemical anchor in the host (von Brand, 1973). In the present study the cholinesterase was demonstrable in regions such as the tegument and the gastrodermis which were also the sites of acid and alkaline phosphatases. It is possible that the enzyme may be involved also in osmoregulation and the permeability of the membranes, and might facilitate the passage of nutrient molecules across these surfaces. Studies on hydatid cyst wall in *Echinococcus* have also shown that acetylcholinesterase is concerned with permeability control and osmoregulation (Schwabe, 1959; Schwabe *et al.*, 1961).

The electrophoretic patterns of esterase isoenzymes are found to vary considerably in parasites from different hosts and different habitats of the same host. For example, *I. hypselobagri* from the swim bladder exhibits five isoenzymes, whereas, *G. crumenifer* and *G. explanatum* which inhabit different regions of the same host contain three and four isoenzymes, respectively.

Further studies on the relative efficiency of the esterases of the parasite with different substrates and the effect of various inhibitors on their activity is necessary in order to make more precise biochemical characterization of the enzyme. Moreover, esterases of the parasite and host tissue have been found to differ in their electrophoretic as well as biochemical properties (Pantelouris, 1967). Comparative study of the esterases of the parasite and its host tissue would also be of great importance for the selection of compounds to be used as anthelmintic drugs.

Summary

Histochemical localization and isoenzyme patterns of esterases by polyacrylamide disc gel electrophoresis are carried out in three species of digenetic trematodes, *Isoparorchis hypselobagri* from the catfish, *Wallago attu*, *Gigantocotyle explanatum* from the liver and *Gastrothylax crumenifer* from the rumen of the water buffalo, *Bubalus bubalis*.

Esterases are demonstrated in the tegument, suckers, gastrodermis, reproductive system, muscular tissue and the nervous system. Inhibition study with eserine

sulfate indicates that cholinesterase is predominantly present in the nervous system. A role of the enzymes in membrane permeability and osmo-regulation is discussed. Electrophoresis reveals that esterases in these parasites occur in isoenzymic forms. The intensities and the electrophoretic patterns of the various isoenzymes are found variable.

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数種二世吸虫類のエステラーゼ活性に関する研究

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Wallago attu (ナマズの一種) の鰾に寄生する *Isoparorchis hypselobagri*, 水牛の肝に寄生する *Gigantocotyle explanatum* 及び同宿主の第1胃に寄生する *Gastrothylax crumenifer* の三種吸虫のエステラーゼについて組織化学及びディスク電気泳動によるアイソザムを検討した, 得られた結果は以下の通りである.

本酵素活性は上記三種吸虫の表は, 吸盤, 腸上皮, 生殖器, 筋組織及び神経系に存在した. エゼリンによる阻害実験により, コリンエステラーゼ活性はこれら吸虫の神経系に主に存在することが判明した.

膜透過及び滲透圧制禦の機構における本酵素の役割についても考察をした.

電気泳動により前記三種の吸虫には 3〜5 のアイソザイムがそれぞれ確認された. これらアイソザイムの活性の強さ及び泳動パターンは吸虫種により異っていた.

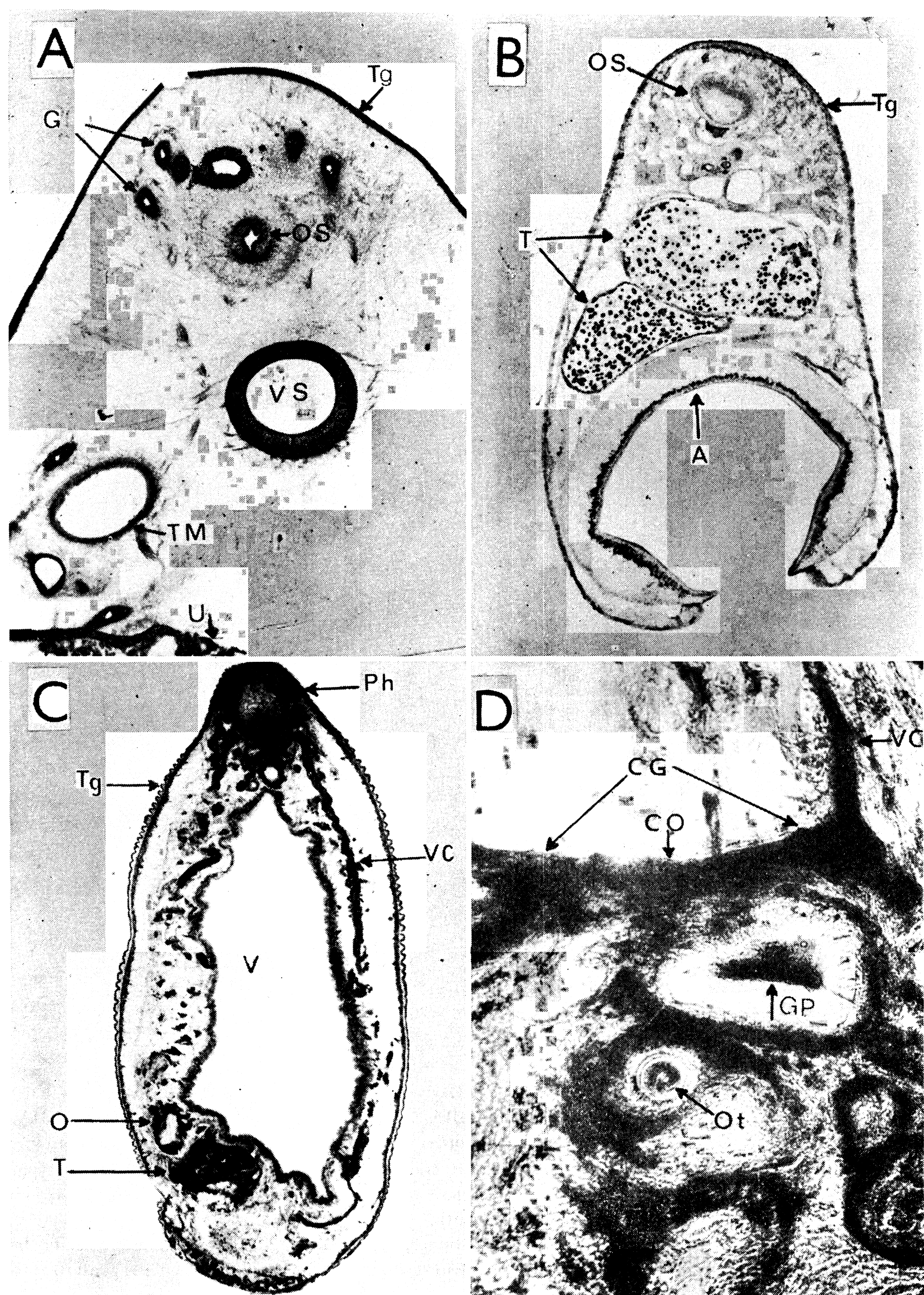

Fig. 1.

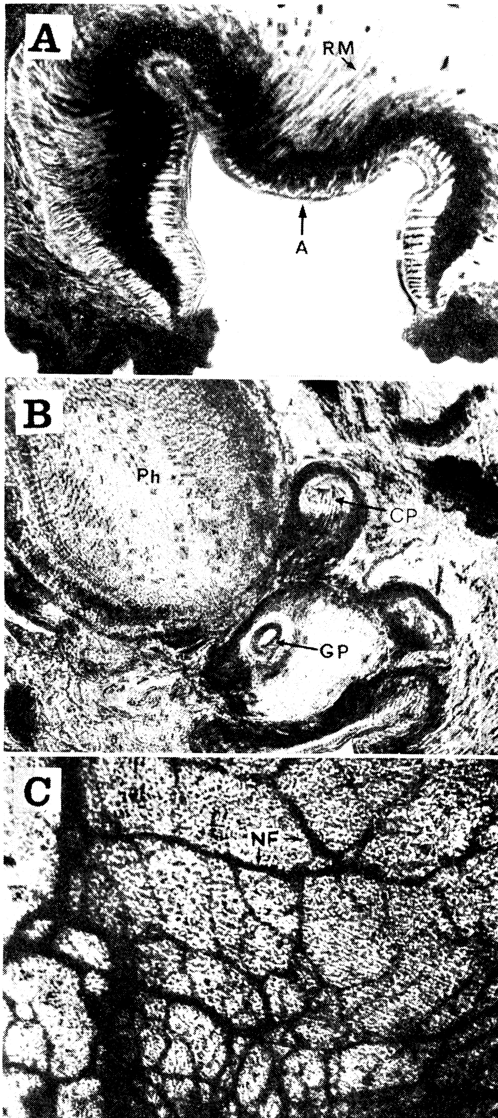

Fig. 2.

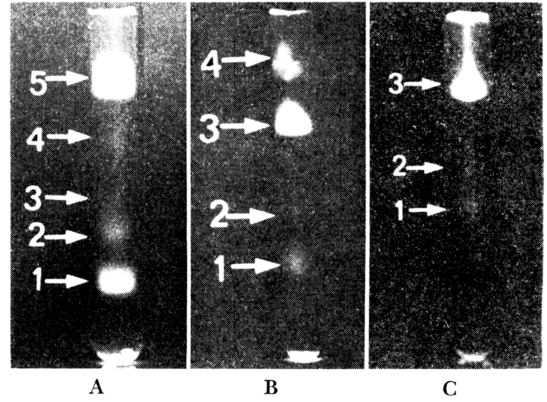

Fig. 3.

Fig. 1 A-D. Frontal sections showing esterase activity. A. *Isoparorchis hypselobagri* (anterior half) 5-BIA, ×10; B. *Gigantocotyle explanatum* 5-BIA, ×10; C. *Gastrothylax crumenifer* 5-BIA, ×10; D. Part of the anterior region of *Gastrothylax crumenifer* 5-BIA, ×50. (A-acetabulum, CG-cerebral ganglion, CO-commissure connecting the ganglia, GP-genital pore, G-gut, Ot-ootype, OS-oral sucker, O-ovary, Ph-pharynx, Tg-tegument, T-testis, TM-testicular membrane, U-uterus, VC-ventral nerve cord, VS-ventral sucker; V-ventral cavity.

Fig. 2 A-C. Frontal sections of *Gastrothylax crumenifer*, 5-BIA, ×50. A. Esterase activity in acetabulum (A) and radial muscles (RM). B. Esterase activity in cirrus pouch (CP), genital pore (GP) and pharynx (Ph).

Fig. 3 A-C. Electrophoretic patterns of esterase isoenzymes. A. *Isoparorchis hypselobagri*, B. *Gigantocotyle explanatum*, C. *Gastrothylax crumenifer*.