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Comparative Studies on Non-Specific Phosphomonoesterases, Glycogen and Pyruvic Acid in *Isoparorchis hypselobagri* from Air Bladder and Body Cavity of *Wallagonia attu*

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Introduction

Non-specific phosphomonoesterases (acid and alkaline phosphatases) play a significant role in a number of metabolic processes, especially carbohydrate metabolism and phosphorylated transfer mechanisms. Von Brand (1973) reviewed the work on non-specific phosphomonoesterases in trematodes. Nizami *et al.* (1975) have studied alkaline phosphatase system in eight species of digenetic trematodes in different hosts and habitats. The present investigation deals with a comparative view of the enzymatic properties of phosphatase system of *Isoparorchis hypselobagri* recovered from air bladder and body cavity of *Wallagonia attu*. Reserve glycogen and pyruvic acid in relation to different habitats have also been studied.

Materials and Methods

Mature worms were recovered from air bladder and body cavity of naturally infected 38 *W. attu* (out of 75 examined). They were washed thrice in distilled water, blotted dry on Whatman paper. Fresh weight were taken for the study of acid and alkaline phosphatases and pyruvic acid. The worms were dried in an oven at 80 C, upto constant weight, for glycogen estimation.

Fresh worms (10% w/v) were homogenised by Remi glass homogenizer (L 563.1 type) in ice cold 0.9% saline, centrifuged at 3,500 rpm for 20 minutes and supernatant was taken for the study of acid and alkaline phosphatases. Phosphatase activity was assayed by the method as described by Wooton (1974), using disodium phenyl-phosphate as substrate. Enzyme activity is expressed in terms of liberated μg phenol/mg protein/hour at 37 C. The protein content of homogenate was determined, following the method of Lowry *et al.* (1951), using bovine serum albumin as standard. In a total volume of 6.4 ml, the reaction mixture contained: 1 ml citrate or carbonate buffer, 1 ml of 0.01 M disodium phenylphosphate, 0.4 ml of 10% (w/v) homogenate of parasite, 0.8 ml of 0.5N NaoH, 1.2 ml of 0.5 N $NaHCo_3$, 1 ml of 0.6% 4-amino antipyrine and 1 ml of 2.4% potassium ferricyanide. Different pH levels, ranging from 2.5 to 11.0 (Citrate and carbonate buffer for acid and alkaline pH respectively), were taken to see the effect of pH on phosphatase activity. Relative activity and effect of metallic ions on acid

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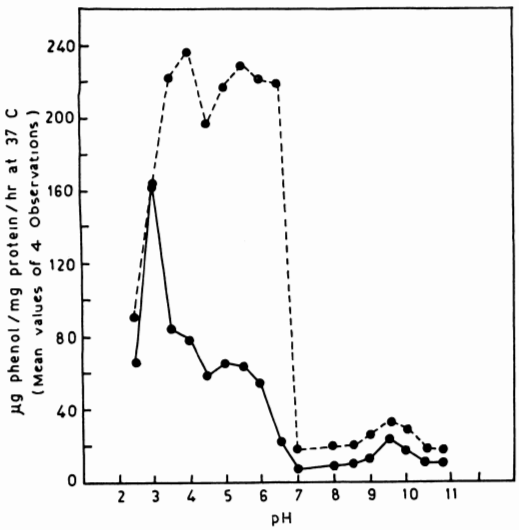

Fig. 1 Effect of pH on acid and alkaline phosphatases of *I. hypselobagri* from air bladder (●——●) and body cavity of (●---●) *W. attu*.

and alkaline phosphatases were carried out at pH 5.0 and 10.0 respectively. The concentration of metallic ions were kept 10^{-3} M in reaction mixture to see the effect of ions on enzyme activity. 50 mg of fresh worms were homogenised by Remi glass homogenizer (L 563.1 type) in distilled water and 10% TCA (1:1) and centrifuged at 3,000 rpm. Supernatant was taken for pyruvic acid estimation by the method of Friedman and Haugen (1943) as described by Oser (1965). Glycogen was estimated on dry weight basis by the method of Seifter *et al.* (1958).

Results

Effect of pH on phosphatase activity is shown in Fig. 1. Maximum activity of acid phosphatase were observed at pH 3.0 and between 5.0 and 5.5 in air bladder form and at pH 4.0 and 5.5 in body cavity form. Maximum activity of alkaline phosphatase was at pH 9.5 in both forms.

Effect of ions on phosphatase activity are summarized in Table 1. Acid phosphatase was activated by Ni^{++} in air bladder form and by Ni^{++} and Co^{++} in body cavity form. Zn^{++} and Cu^{++} considerably activated alkaline phosphatase in air bladder form, but inhibited the same in body cavity form, Hg^{++} activated alkaline phosphatase of body cavity form but has no effect on the same in air bladder form. Further, it inhabited acid phosphatase in both forms. Mn^{++}, Hg^{++}, Co^{++}, Ni^{++} and NaF have no effect on alkaline phosphatase of air bladder forms. Similarly, Mn^{++} and Mg^{++} have no effect on acid phosphatases of body

Table 1 Effect of ions* on acid and alkaline phosphatases in *I. hypselobagri* from air bladder and body cavity of *W. attu*.

Ions	Air bladder form		Body cavity form	
	ACPase (%)	ALKPase (%)	ACPase (%)	ALKPase (%)
Mn^{++}	−23.06	0	0	−100.00
Hg^{++}	−38.44	0	−55.55	+ 14.25
Zn^{++}	−92.24	+149.91	−16.66	−100.00
Cu^{++}	−38.44	+ 49.97	−22.22	− 92.70
Co^{++}	− 7.68	0	+ 5.56	− 85.56
Ni^{++}	+76.91	0	+38.89	− 64.17
Mg^{++}	−15.36	− 75.02	0	− 92.70
V^{+5}	−92.24	− 75.02	−83.33	−100.00
Fe^{+3}	−92.24	− 25.04	−88.89	− 92.70
NaF	−92.24	0	−94.45	− 57.06
EDTA	− 7.68	− 75.02	+22.22	− 85.56

−= Inhibition, += Activation, *= Ionic concentration in reaction mixture was 10^{-3}M.

Table 2 Relative activity of acid and alkaline phosphatases, glycogen and pyruvic acid in *I. hyposelobagri* from air bladder and body cavity af *W. attu*

		Air bladde r form	Body cavity form
I.	Relative activity (μg phenol/mg/protein/hr)		
	Acid phosphatase (pH 5.0)	18.43±2.87 (N*=8)	86.05±3.86 (N =9)
	Alkaline phosphatase (pH 10.0)	14.75±1.44 (N =8)	23.37±0.95 (N =9)
II.	Glycogen (% on dry weight basis)	22.19±6.97 (N =13)	15.84±2.55 (N =11)
III.	Pyruvic acid (μg/100 mg fresh tissue)	6.24±1.28 (N =5)	18.12±3.75 (N =5)

N*= The number of determination.

cavity form. V^{+5} and Fe^{+3} markedly inhibited phosphatases in both forms. EDTA uniformly inhibits enzyme activity except acid phosphatase in body cavity form. Extent of activation and inhibition by different ions also differed in the two forms.

Results obtained from relative activities of non-specific phosphomonoesterases, glycogen and pyruvic acid are summarized in Table 2. Relative activity of non-specific phosphomonoesterases was found higher in body cavity form. Acid phosphatase activity was 18.43±2.87 and 86.05±3.86 and alkaline phosphatase activity was 14.75± 1.44 and 23.37±0.95 in air bladder and body cavity forms respectively.

Reserve glycogen was found more in air bladder form (22.19±6.97) than in body cavity form (15.84±2.55). Pyruvic acid was found higher in body cavity form (18.12± 3.75 μg/100 mg) than in air bladder form (6.24±1.28 μg/100 mg). Differences observed were found statistically significant.

Discussion

The activity of acid phosphatases was found higher than alkaline phosphatase in both forms. Higher activity of acid phosphatases in *I. hypselobagri* agrees with the observations of earlier investigators (Pennoit-De Comman and Van Grembergen, 1942 in *Fasciola hepatica*; Nimmo- Smith and Standen, 1963 in *Schistosoma mansoni*; Ma, 1964 in *Clonorchis sinensis*; Goil, 1966 in *Gastrothylax crumenifer*; Halton, 1967 in eight species of trematodes; Probert and Lwin, 1974 in *F. hepatica*; Sathyanarayana and Anantaraman, 1979 in *Ganeo tigrinum* and Hora and Sharma, 1980 in *Ceylonocotyle scoliocoelium* and *Paramphistomum cervi*). It is, therefore, evident that a higher level of acid phosphatases exists in all the adult trematodes so far studied.

The present authors have observed only one peak at pH 9.5 for alkaline phosphatase activity. Nizami *et al.* (1975) reported three pH optimas at 8.0, 9.0 and 10–10.5 respectively. Since the substrates, units of activity and the method used are different, it is difficult to compare the biochemical results of the present study with those of the previous workers.

The twoforms showed different pH optimas for acid phosphatase (viz. 3.0 and 5.0–5.5 in air bladder form and 4.0 and 5.5 in body cavity form), while pH optimum for alkaline phosphatase was found to be identical (pH 9.5). Two habitats, air bladder and body cavity, differ widely in availability of food and oxygen and thus exert profound influence on physiological processes, particularly carbohydrate metabolism. Acid phosphatases play a major role among non-specific phosphomonoesterases in trematodes, thus the differences observed in respect of acid phosphatases may be due to different habitat. This

agrees well with Nizami *et al.* (1975) who, in their study of alkaline phosphatases in eight different trematodes, attributed difference in pH optima to different habitats.

Nizami *et al.* (1975) reported activation of alkaline phosphatases by Mg^{++} and Co^{++}. In the present study, however alkaline phosphatase activity was inhibited by Mg^{++} in air bladder form while in body cavity form it has no effect. Further, Co^{++} activated the alkaline phosphatase in body cavity form whereas inhibition was observed in air bladder form. Inhibition of enzyme activity by NaF and EDTA suggests that both enzymes are metalloenzymes. Effect of metallic ions on phosphomonoesterases in trematodes are scanty, hence it is difficult to interpret the data obtained in the present study.

Acid and alkaline phosphatases of a species of worms parasitizing different habitats in the same host species have not been studied before. The present authors have found significantly high phosphatase activity in body cavity form as compared from that in air bladder form of *I. hypselobagri*. Since host species, developmental stage of worms and experimental conditions were identical, this difference in relative activity of phosphatases may be due to the differences in habitats. Higher activity of phosphatases in body cavity forms suggests that they are metabolically more active. This seems cogent because body cavity is an unusual habitat, hence worms inhabiting the same are under stress. Lesser reserve glycogen and relatively high pyruvic acid in body cavity forms, as compared with air bladder forms, substantiate this.

Summary

Isoparochis hypselobagri is found to occur both in air bladder and body cavity of *Wallagonia attu*. While total glycogen content is more in air bladder form, pyruvic acid is found more in body cavity form. Activity of non-specific phosphomonoesterases (acid and alkaline phosphatases) is higher in body cavity form. Maximum activity of alkaline phosphatase was at pH 9.5 in both forms; acid phosphatases, on the other hand, showed two peaks at pH 3.0 and between 5.0 and 5.5 in air bladder form, and at pH 4.0 and 5.5 in body cavity form. Extent of activation and inhibition of acid and alkaline phosphatase activities in air bladder and body cavity forms varied considerably with different ions.

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References

1) Friedman, T. E. and Haugen, G. E. (1943): As cited by Oser in Hawk's Physiological Chemistry, 14th ed. (New Delhi- Tata McGraw-Hill 1965), 1108.
2) Goil, M. M. (1966): Physiological studies on trematoda: Phosphatases system in *Gastrothylax crumenifer*. Parasitol., 56, 105–110.
3) Halton, D. W. (1967): Studies on phosphatase activity in trematoda. J. Parasitol., 53, 46–54.
4) Hora, C. and Sharma, P. N .(1980): Comparative biochemical observation on glycogen, protein, lipids and enzyme of *Ceylonocotyle scoliocoelium* and *Paramphistomum cervi*, the rumen amphistomes of Buffalo. Proc. Indian Acad. Parasitol., 1, 125–129.
5) Lowry, O. H., Roserbrough, N. J., Farr, A. L. and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265–275.
6) Ma, L. (1964): Acid phosphatases in *Clonorchis sinensis*. J. Parasitol., 50, 235–240.
7) Nimmo-smith, R. H. and Standen, O. D. (1963): Phosphomonoesterases of *Schistosoma mansoni*. Exp. Parasitol., 13, 305–322.
8) Nizami, W. A., Siddiqui, A. H. and Yusufi, A. N. K. (1975): Non-specific alkaline phosphomonoesterases of eight species of digenetic trematodes. J. Helminthol., 49, 281–287.
9) Oser, B. L. (1965): Hawk's Physiological Chem-

istry. 14th ed, Tata McGraw Hill book company, New Delhi, 1472.
10) Pennoit-De Cooman, E. and Van Grembergen, G. (1942): Vergelikend onderzoek van het fermenten system bij vrijlevende en parasitaire Plathelminthen, Vol. IV, 7–77. Verslagen en voorstellen van de kvlaanse Academic voor Wetenschappen, leheren en schone kunstern van Belgie, Brussels.
11) Probert, A. J. and Lwin, T. (1974): Kinetic properties and location of non-specific phosphomonoesterases in subcellular fraction of *Fasciola hepatica*. Exp. Parasitol., 35, 253–261.
12) Sathyanarayana, M. C. and Anantaraman, S. (1979): Non-specific phosphomonoesterases of *Ganeo tigrinum* Mehra and Negi, 1928. (Trematoda). Proc. Indian Acad. Sci., 88 B, 1, 115–118.
13) Seifter, S., Daytons, S., Novic, B. and Muntwyter, E. (1950): The estimation of glycogen with Anthrone reagent. Arch. Biochem., 25, 191–200.
14) Von Brand, T. (1973): Biochemistry of Parasites. 2nd ed., Academic Press, New York and London, 499.
15) Wootton, I. D. P. (1974): Microanalysis in Medical Biochemistry. 5th ed., Churchill Livingstone, Edinbury and London, 307.

Wallagonia attu の鰾および体腔に寄生する *Isoparorchis hypselobagri* 成虫の非特異的フォスフォモノエステラーゼ活性，グリコーゲン，ピルビン酸量について

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Wallagonia attu（ナマズの一種）の鰾および体腔など寄生部位を異にする吸虫 *Isoparorchis hypselobagri* 成虫の酸およびアルカリフォスファターゼ活性，グリコーゲン量，およびピルビン酸量を比較検討した．

両種フォスファターゼの比活性は体腔寄生(体腔型)の成虫で鰾寄生（鰾型）のそれよりも高い値を示す．鰾型成虫の酸フォスファターゼの至適 pH は 3.0 と 5.0～5.5，体腔型のそれは 4.0 と 5.5 である．アルカリフォスファターゼの至適 pH は両型ともに 9.5 である．

鰾型の酸フォスファターゼ活性は Ni^{++} で，体腔型のそれは Ni^{++} と Co^{++} の両イオンで活性上昇が見られる．鰾型のアルカリフォスファターゼ活性は Zn^{++} と Cu^{++} で活性上昇するが，体腔型のそれでは活性阻害が見られる．Hg^{++} は体腔型アルカリフォスファターゼの活性を上昇させるが鰾型のそれには効果がない．又 Hg^{++} は両型の酸フォスファターゼ活性を阻害する．

Mn^{++}, Hg^{++}, Co^{++}, Ni^{++} および NaF は鰾型アルカリフォスファターゼ活性に対し無効である．同様 Mn^{++} および Mg^{++} は体腔型酸フォスファターゼ活性に無効である．V^{+5} 及び Fe^{+3} は両型両種のフォスファターゼ活性を阻害し，EDTA は体腔型酸フォスファターゼ以外のすべてのフォスファターゼ活性を阻害する．各種イオンによるフォスファターゼ活性の阻害および賦活の程度は寄生部位の異なる成虫の間で夫々異なる．

グリコーゲン量は鰾型成虫においてより多量に含有するも，ピルビン酸量は体腔型成虫でより多量に含まれる．上記各種の生化学的数値の両型成虫間の差は統計学上有意のものである．