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Activity of Some Hepatic Enzymes in Schistosomiasis and Concomitant Alteration of Arylsulfatase B

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The levels of arylsulfatases A and B, α-amylase, aspartate transcarbamylase, and γ-glutamyl transpeptidase were investigated during the infection of mice with *schistosoma mansoni*. This infection caused a significant ($p<0.001$) increase in the activity of hepatic arylsulfatase B (ASB), aspartate transcarbamylases and γ-glutamyl transpeptidase. A non-significant difference occurred for α-amylase ($p<0.3$) and arylsulfatase A ($p>0.5$) when compared to the control. The specific activity of hepatic ASB was progressively increased with the progression of the *Schistosoma*-infection. Moreover, the kinetic studies of hepatic ASB in *Schistosoma*-infection showed that a slight decrease in the value of K_m and about a 40% increase in V_{max} when compared to the control. In addition, the pH optimum of hepatic ASB was altered from 6 to 7 as a result of schistosomiasis. These observations suggest that there are schistosomiasis-associated changes of the catalytic and kinetic properties of hepatic ASB.

Keywords: Enzyme activity, Kinetic parameters, Schistosomiasis

Introduction

Schistosomiasis is a chronic worm infection that is transmitted through contact with fresh water, which has been infested with certain species of aquatic snails (Esrey *et al.*, 1991; Shiff *et al.*, 1993). The schistosomes or blood flukes are the most clinically significant trematode parasites. The infection may occur in one of several species: *Schistosoma hematobium*, *Schistasoma mansoni*, *Schistosoma Japonicum*, etc (Larotski and Davis, 1981). This endemic disease affects the liver, intestine, spleen, and urinary bladder (Mellin *et al.*, 1993). However, various enzyme changes were observed during *Schistasoma mansoni*, such as arylsulfatase B (Higuchi *et al.*, 1984) and many hydrolases (Nabih and El-Ansary, 1991; Cesari *et al*., 2000; Balbaa *et al.*, 2003).

The α-amylases (EC 3.2.1.1) are endogluconases that catalyze the hydrolysis of internal α-1, 4 glucosidic linkage in starch and related polysaccharides (Robyt and Frensh, 1970). Arylsulfatases A, B, and C (arylsulfo-hydrolases) are a group of hydrolytic enzymes that occur in various tissues and fluids (Rockfor *et al*., 1976). Arylsulfatase A (ASA, EC 3.1.6.8) is a cerebroside-3-sulfohydrolase. It hydrolyses galactose-3-sulfate residues in cerebroside sulfate and other sulfated galactolipids (Boyland *et al.*, 1955; Wasserman and Austen, 1976; Poys and Morgan, 1977). Arylsulfatase B (ASB, EC 3.1.6.9) is a lysosomal hydrolase that desulfates the non-reducing terminal N-acetylgalactosamine-4-sulfate residues that are present in glycosaminoglycan (Rockfor *et al.*, 1976). This sulfatase is usually a basic protein and easily separated from ASA by ion-exchange chromatography (Wasserman and Austen, 1976). ASB activity is increased in bladder tumors (Boyland *et al.*, 1955; Poys and Morgan, 1977) and colorectal carcinoma (Morgan *et al.*, 1975). In visceral neoplasm, the ASB activity is either elevated or depressed in a tissue specific manner (Dzialoszynski *et al.*, 1966; Morgan *et al.*, 1972). Aspartate transcarbamylase (ATCase, EC 2.1.3.2) catalyzes the carbamaylation of the α-amino group of L-aspartate by carbamyl phosphate (CP) to yield carbamyl aspartate and inorganic phosphate. In eukaryotes, the activities of CP synthetase, ATCase, and dihydrooratase (the enzymes which catalyze the first three steps in pyrimidine biosyntheses) were reported to be associated in a multi-enzyme complex (Mori *et al.*, 1975; McKee and McKee, 2003). On the other hand, γ-

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glutamyltranspeptidase (GGT, EC 2.3.2.2) is a membrane-bound enzyme that catalyzes the transfer of the γ-glutamyl moiety of glutathione to an amino acid or a peptide, hydrolysis of glutathione and autotranspeptidation (Meister, 1973).

The present study was undertaken to look for some biochemical changes and an enzyme marker for the infection by *Schistosoma mansoni*. Different types of enzymes (such as α-amylase, arylsulfatases A and B, ATCase, and GGT) were investigated in an attempt to detect an enzyme that might be disease-specific (as sensitive as possible) and to reflect changes occurring in the liver in the early phases of the disease progression.

Materials and Methods

Chemicals *p*-Nitrocatechol sulfate (PNCS), dilithuim carbamyl aspartate, dinitrosalicylic acid, γ-glutamyl-*p*-nitroanilide, Concanavalin A (Con A)-Sepharose, and bovine serum albumin were purchased from Sigma Chemical Co. (St Louis, USA). DEAE-cellulose (DE52) was purchased from Whatman (Maidstone, UK). Gly-Gly and glutathione were a kind gift from the Department of Biochemistry, Faculty of Medicine, University of Toronto, Canada. Folin-ciocalteu, phenol reagent, and other chemicals were purchased from Merck (Whitehouse Station, USA). All of the other reagents were of analytical grade.

Animals Four-week old male Swiss albino mice (CDI) with an average weight of 15-20 g were obtained from the Theodore Bilharz Research Institute, Schistosome Biological Supply Program (SBSP), Cairo, Egypt. The mice were housed in wire cages in groups of 5 mice per cage. They were kept under conventional conditions of temperature and humidity with a 12 h-photoperiod. Food and water were supplied ad libitum.

Animal infection Seventy mice were infected with *Schistosoma mansoni* by an intrapretoneal injection of an average of 80 cercariae /mice. The infected mice were grouped (6-25 mice per group) according to the duration of infection with a 15 d-interval between each group. Another seventy mice were used as the control and were divided into groups (5-25 mice per group), corresponding to the infected groups. At the end of each infection period, the infected group and the corresponding control one were sacrificed. The livers were immediately removed, weighed, rinsed with chilled saline solution, and homogenized in 9 volumes of ice-cold 10 mM Tris-HCl buffer, pH 7.4, containing Triton X-100 and 0.25 M sucrose using a Brinkman homogenizer. The homogenate was centrifuged at $500 \times g$ and 4°C for 20 min. The obtained supernatant was subjected to enzyme assay.

Enzyme assay For arylsulfatases, the $500 \times g$ supernatant was applied on a DEAE-cellulose column (5×1 cm), equilibrated, and washed with a 10 mM Tris-HCl buffer, pH 7.4. ASB was obtained in the pass through and washing fractions. ASA was eluted with 5 volumes of the same buffer containing gradient concentrations of sodium chloride (0.0-0.5 M). The activities of ASA and ASB were assayed according to the colorimetric method of Baum *et al.* (1958) and Baum and Dodgson (1958) using PNCS as a substrate at 37°C. One unit of enzyme activity is defined as one nmol of *p*-nitrocatechol liberated per an hour under the assay condition. For ATCase, the activity was assayed, as previously described (Balbaa *et al.*, 2001), in 12.5 mM aspartate and 3.6 mM dilithium carbamyl phosphate at pH 8.2 and 30°C. For α-amylase, the saccharogenic enzyme activity was determined according to the method of Fischer and Stein (1961) using 3,5-dinitrosalicylic acid as a substrate at 37°C. For GGT, the transpeptidation assay of GGT was conducted as previously described (Minato 1978; McIntyre and Curthogs 1979). The assay contained 2.5 mM γ-glutamyl-*p*-nitroanilide, 0.1 M HCl, 0.1 M $MgCl_2$, and 0.1 M Gly-Gly in 1 M Tris-HCl buffer, pH 9.0, at 37°C.

Purification of ASB The enzyme purification from the livers of normal and infected mice was carried out at 4°C, as previously described by Balbaa (1988). They include ammonium sulfate precipitation of $20{,}000 \times g$ supernatant from the whole homogenates and chromatographies on DE52, Con A-Sepharose, and Sephadex G-150 columns.

Kinetic studies The kinetic parameters (K_m and V_{max}) of ASA and ASB were determined from a Lineweaver-Burk plot of 1/[S] against 1/v. The substrate concentration ranges were from 0.3 to 10 mM and from 15 to 50 mM for ASA and ASB, respectively. The heat stability of the purified ASB was studied by its incubation in a 10 mM Tris- HCl buffer, pH 7.4, for 10 min at various temperatures (10-80°C). The enzyme activity was assayed at 37°C. The pH profile was studied for the purified ASB by its assay at different pH values from 2 to 8 at constant substrate and enzyme concentrations.

Protein assay The protein concentration was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Statistical analyses The comparison between the control and infection was made by a student's t-test. Data were expressed as mean ± S.E. Statistical significance was identified at $p<0.05$. All of the experiments were run on three occasions for reproducibility, and all of the assays were done in triplicate.

Results

Table 1 shows the effect of the *Schistosoma* infection on the specific activities of hepatic ASA, ASB, α-amylase, ATCase, and GGT. The activities of hepatic ASB, ATCase, and GGT showed a significant change ($p<0.001$) in the infected mice when compared to the control. A significant increase was observed for ASB, ATCase, and GGT. The α-amylase and ASA in the livers of the infected mice displayed a non-significant decrease, whereas $p<0.3$ and $p<0.5$ were observed for their activities when compared to the control, respectively. The effect of different periods of *Schistosoma* infection on the specific activity of the investigated enzymes showed that hepatic ASB is successively increased when the infection

Table 1. Specific activities of hepatic arylsulfatases A and B, α-amylase, ATCase, and GGT from *Schistosoma*-infected mice compared to control

Enzyme	Specific Activity		P value
	Control	Infection	
ASA	0.58±0.07[a] (5)	0.65±0.04[a] (6)	>0.500
ASB	72±13[a] (6)	107±14[a] (6)	<0.001
α-amylase	4.7±1.6[b] (25)	2.5±1.4[b] (25)	<0.300
ATCase	208±7.0[c] (10)	260±11[c] (12)	<0.001
GGT	1,050±170[d] (7)	1,410±220[d] (12)	<0.001

Mice were infected by 80 cercariae for 60 days and subjected to the same conventional conditions of control.
[a]nmoles/h/mg protein, [b]mU/mg protein, [c]nmoles/30 min/mg protein, [d]nM/min/mg protein.

Table 2. Purification steps of ASB from liver of infected mouse and control

Step	Specific activity (nmol/h/mg protein)	Fold	Yield (%)
Whole homogenate	56 (80)	1.00	100
DE-52 (unadsorbed fraction)	94 (144)	1.7 (1.8)	49 (48)
Con A-Sepharose eluate	10,200 (9,540)	183 (120)	33 (49)
Sephadex G-150	100,840 (140,100)	1,816 (1,760)	20 (34)

The values for the enzyme in infection are shown between brackets.

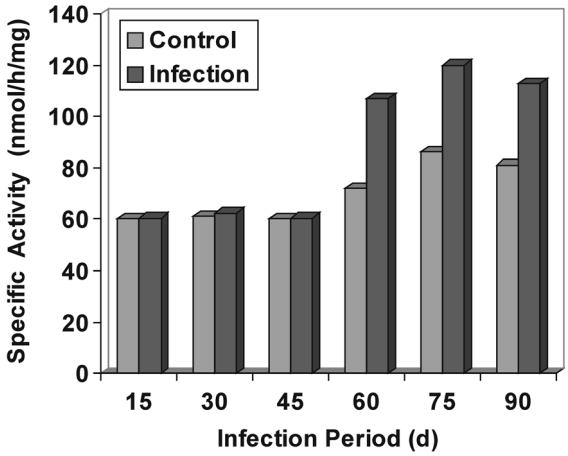

Fig. 1. Specific activity of hepatic ASB from mice during *Schistosoma* infection. The specific activity was measured at 37°C and pH 6.0 within different intervals of infection (15-90 d).

interval is increased. As graphically illustrated in Fig. 1, hepatic ASB showed a significant elevation of its specific activity at 60, 75, and 90 d post infection with *Schistosoma mansoni*. At these periods, the specific activities of 107 ± 14, 120 ± 25, and 113 ± 9 nmol/h/mg were observed when compared to the values of 72 ± 13, 86 ± 17, and 81 ± 1 nmol/h/mg for the control, respectively.

ASB was separated from the mice liver by different steps. As shown in Table 2, ASB from the control and infected mice was purified about 1,816-fold and 1,760-fold, respectively, beginning with the crude homogenate and continuing to the final step. The post-gel filtration fraction retained approximately 20 and 34% of the original total activity (yield) for the enzyme from the control and infected mice, respectively.

Table 3. The kinetic parameters of hepatic arylsulfatases from *Schistosoma*-infected mice compared to control

Parameter	Control		Infection	
	ASA	ASB	ASA	ASB
K_m (mM)	1.0	3.9	0.5	3.2
V_{max} (nmol/min/mg)	3.0	137	3.2	227

The infection occurred by 80 cercariae for 90 days. All reactions were performed at 37°C. ASA and ASB were assayed at pH 5.0 and 6.0, respectively.

Kinetic studies Table 3 shows the kinetic parameters of hepatic ASA and the purified hepatic ASB from the *Schistosoma*-infected mice when compared to the control. The K_m value of ASA toward PNCS as a substrate is 1.0 and 0.5 mM in the control and infected mice, respectively. The enzyme from the control and infected mice had V_{max} values of 3.0 and 3.2 nmol/h/mg protein, respectively. For ASB, K_m of the enzyme toward PNCS was found to be 3.9 and 3.2 mM in the control and infected cases, respectively. The observed value of V_{max} was increased and was equal to 137 and 227 nmol/min/mg protein for the enzyme from the control and infected mice, respectively.

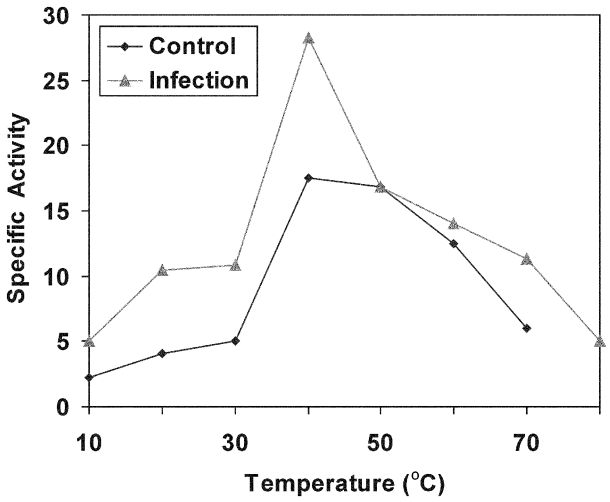

Fig. 2. Heat stability of hepatic ASB from control and *Schistosoma*-infected mouse. Specific activities as nmol/h/mg protein were measured at 37ºC and pH 6.0 after treatment of the enzyme by different temperature values (10-80ºC).

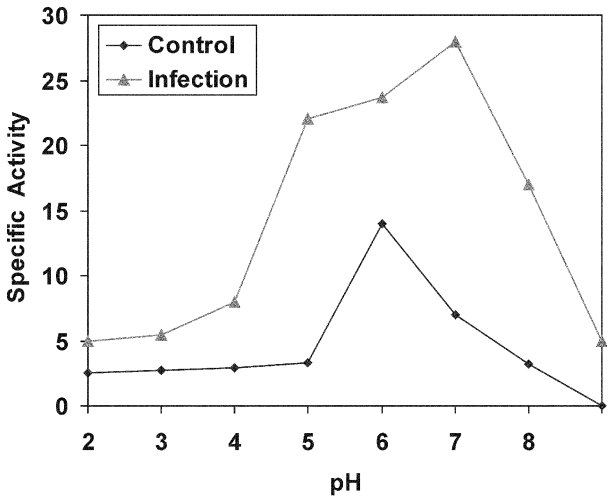

Fig. 3. pH profile of hepatic ASB from control and *Schistosoma*-infected mouse. Specific activity, expressed as nmol/h/mg protein at 37ºC, was measured at different pH values (2-8).

Heat stability Figure 2 illustrates the pattern of temperature effect on the specific activity of hepatic ASB from the control and infected mice at pH 6.0. It is clear that the enzyme from the infected mice was relatively more stable than that of the control, especially at 20ºC and 30ºC. The enzyme from the infected mice showed more stability than that of the control at 70ºC.

pH profile The specific activity of ASB from the control and infected mice, measured at different values of pH, is illustrated in Fig. 3. The pH optimum for the enzyme in the control and infected mice (Fig. 3) was 6.0 and 7.0, respectively. The enzyme from the infected mice, when compared to the control (Fig. 3, upper line), displayed a relatively broad profile.

Discussion

In the present study, schistosomiasis is considered an important disease to investigate, because of its effect on some enzymes that have important roles in the cell. These include ASA, ASB, α-amylase, ATCase, and GGT. ASA and ASB, which are the two components of arylsulfatases, were fractionated by ion-exchange chromatography (Wasserman and Austen, 1976) and assayed by PNCS as the same specific substrate (Baum *et al.*, 1958; Baum and Dodgson, 1958). The results of the present study demonstrate that ASA exhibited no significant change in its activity in the livers of the infected mice when compared to the control, while ASB showed a highly significant increase in its activity. In addition, ASB activity was clearly increased with the progression of the *Schistosoma* infection. This finding is consistent with previous reports for the *Schistosoma* infection of humans (Khafagy *et al.*, 1979).

Metabolic and enzyme changes have been observed in the *Schistosoma* infection. Disturbances in carbohydrate metabolism were obvious in the liver homogenate of *Schistosoma*-infected mice, as represented by a decrease in the activity of glucose-6-phosphatase (El-Merzabani *et al*., 1977). This shows the damaging effect of schistosomiasis on liver parenchyma cells, which leads to the inability of the liver to dephosphorylate glucose-6-phosphate into glucose (El-Aaser *et al.*, 1989; Zakhary, 1994). A marked reduction in the ATPase activity, and conversely a noticeable elevation in the activity of alkaline phosphatase, were observed in the livers of the *Schistosoma*-infected mice (Emmelat and Bos, 1971). Moreover, infection with *Schistosoma mansoni* resulted in a disturbance in the nucleic acid metabolism in the livers of infected mice (Hunter *et al.*, 1973). Recently, it has been found that lipase, α-amylase, and leucine aminopeptidase displayed a significant increase in human serum during schistosomiasis (Balbaa *et al.*, 2003). It was reported that the induction of nitric oxide synthase activity in murine schistosomiasis lead to deleterious effects on the liver (Abdallahi *et al*., 2001). This suggests the involvement of cell signaling during these effects.

However, the significant increase of ATCase in the liver reflects the pyrimidine supply *in vivo* where this enzyme catalyzes the initial step in its biosynthesis (McKee and McKee, 2003). The regulation of this enzyme reaction is very critical for pyrimidine nucleotide biosynthesis (Balbaa *et al.*, 2001; McKee and McKee, 2003). On the other hand, the significant increase of GGT in the liver may be an indication for upper degradation of glutathione and the formation of γ-glutamyl-amino acids, as reported for the role of this enzyme (Meister, 1973).

It was previously reported that ASA from human tissues

has a range of K_m values from 0.2 to 0.6 mM toward PNCS (Baum *et al.*, 1958; Nakamura *et al.*, 1984). While ASB from human livers (Gasa *et al.*, 1987) and lungs (Baum and Dodgson, 1958) has a K_m value of 0.6 and 15.5 mM toward PNCS, respectively. The kinetic study of ASA showed that the K_m value of the enzyme from the infected mice was 0.47 mM, which is half the value of that from the control. This means that a slight change in the enzyme affinity resulted from schistosomiasis, and this paralleled the non-significant change of the activity. *Schistosoma* infection caused a slight decrease in the K_m value of ASB, indicating a slight change in the affinity of ASB due to infection. However, a value of about 66% increase in V_{max} was observed in the infected groups. This indicates a change in the catalytic properties of ASB. This is supported by the change of the pH optimum of the enzyme from pH 6 to 7 for that from the control and infected mice, respectively. Most enzymes are easily unfolded by heating as a result of increased vibrational and rotational motions of atoms (Price and Steven, 1989). In fact, it was reported that ASB is subjected to regulation by reversible phosphorylation that is catalyzed by cAMP-dependent protein kinase, as previously reported in lung cancer (Gasa *et al.*, 1987; Balbaa *et al.*, 1988). However, its activity was changed. In some tumors, ASA and ASB are modified by sialylation and phosphorylation (Gasa *et al.*, 1981; Gasa and Makita, 1983; Uehara *et al.*, 1983; Nakamura *et al.*, 1984). Accordingly, we suggest the possibility of the involvement of a regulation mechanism that can lead to a changed behavior of ASB in schistosomiasis.

In conclusion, the change of the level of the investigated enzymes in the present study represents a basis to approach the biochemical changes in schistosomiasis. The significant change of ASB in *Schistosoma* infection may be related to a change in the catalytic and structural properties. In addition, ASB may be subjected to a regulatory mechanism in the cell during schistosomiasis. However, further studies are required to support this assumption.

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