Comparison of arylsulphatases from *Eimeria tenella* (parasite) and chicken caecum (host)

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Chicken caecal arylsulphatase was purified 700-fold by $(\text{NH}_4)_2\text{SO}_4$ fractionation, concanavalin A–Sephacrose and cyclic AMP–Sephacrose chromatographies. The purified enzyme was a glycoprotein of M_r 97 000. It hydrolysed *p*-nitrocatechol sulphate, cerebroside 3-sulphate and ascorbic acid 2-sulphate and was strongly inhibited by Na_2SO_4 ($K_i = 50 \mu\text{M}$) and Na_3PO_4 ($K_i = 20 \mu\text{M}$). Arylsulphatase from *Eimeria tenella* sporozoites was purified 28-fold by $(\text{NH}_4)_2\text{SO}_4$ fractionation. Arylsulphatase of *E. tenella* sporozoites was not a glycoprotein. It had an M_r of 49 000. It was inhibited by Na_2SO_4 ($K_i = 300 \mu\text{M}$), sodium phosphate ($K_i = 90 \mu\text{M}$) and heparin. It hydrolysed ascorbic acid 2-sulphate, but cerebroside 3-sulphate was not desulphated. The kinetic parameters of chicken caecal arylsulphatase were different from those of the *E. tenella* enzyme.

INTRODUCTION

Arylsulphatases (EC 3.1.6.1), which catalyse the hydrolysis of sulphate esters of various phenolic compounds, are universally distributed, from bacteria to mammals (Dodgson & Rose, 1975; Roy, 1976; Farooqui & Mandel, 1977; Farooqui, 1980, 1987). Three types of arylsulphatases, namely arylsulphatases A, B and C, distinguishable by their relative substrate specificities, response to inhibitors and intracellular localization, have been shown to exist in mammalian tissues (Dodgson & Rose, 1975; Farooqui, 1980, 1987). Arylsulphatase A is involved in degradation of those sulphate esters which contain galactose 3-sulphate, such as cerebroside 3-sulphate (Mehl & Jatzkewitz, 1968; Porter *et al.*, 1972; Farooqui & Bachhawat, 1972, 1973) and seminolipid (Fluharty *et al.*, 1974; Farooqui, 1976a). Arylsulphatase B hydrolyses the sulphate group from *N*-acetylgalactosamine 4-sulphate (Fluharty *et al.*, 1975; Farooqui & Roy, 1976; Farooqui, 1976b). Arylsulphatase C is involved in the degradation of steroid sulphates (Farooqui, 1980, 1981, 1987). Arylsulphatases have been extensively purified and characterized from various mammalian (Nichol & Roy, 1964; Stevens *et al.*, 1975; Farooqui, 1976a; Farooqui & Roy, 1976; McGovern *et al.*, 1982; Moriyasu *et al.*, 1982) and avian (Farooqui & Bachhawat, 1972; Ahmad *et al.*, 1977; Feddecka-Bruner & Croisille, 1978) tissues. However, little is known about these enzymes in intestinal tissues (Hsu & Tappel, 1964; Szafran & Szafran, 1963; Danovitch & Laster, 1969).

During our studies on *Eimeria tenella*, a parasitic protozoan which infects the caecal epithelial cells of domestic chickens and causes the disease caecal coccidiosis (Hammond, 1973), we found that the sporozoites of this parasite contain an arylsulphatase activity (Farooqui *et al.*, 1983). Hence it became important for us to distinguish between host-tissue (chicken caecum) and parasitic (*E. tenella*) arylsulphatases. In the present paper, we describe simple methods for the purification of chicken caecal and *E. tenella* arylsulphatases. Several

properties of chicken caecal arylsulphatase have also been compared with arylsulphatase activity of *E. tenella* sporozoites.

MATERIALS AND METHODS

Materials

p-Nitrocatechol sulphate, *p*-nitrophenol sulphate, cyclic AMP and cyclic AMP–Sephacrose, hyaluronic acid, chondroitin sulphate (mixture of chondroitin 4- and 6-sulphate), and heparin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.; concanavalin A–Sephacrose, a M_r -determination kit and Blue Dextran 200 were obtained from Pharmacia, Uppsala, Sweden. Cerebroside 3-sulphate and ascorbic acid 2-sulphate were prepared as described previously (Farooqui & Bachhawat, 1972; Quadri *et al.*, 1973). Rhodizonate (dipotassium salt) was purchased from Eastman Chemical Co., Rochester, NY, U.S.A. All other chemicals used were of analytical grade.

Assay of arylsulphatases

Chicken caecal arylsulphatase was assayed by measuring the formation of *p*-nitrocatechol from *p*-nitrocatechol sulphate. The assay mixture consisted of 200 mM-sodium acetate buffer, pH 5.5, 4 mM-*p*-nitrocatechol sulphate and up to 50 μg enzyme protein in a total volume of 0.5 ml. The reaction mixture was incubated for 5 min at 37 °C and the reaction was stopped by the addition of 2.5 ml of 0.5 M-NaOH. The amount of *p*-nitrocatechol formed was determined spectrophotometrically at 515 nm. One unit of enzyme activity was defined as the amount liberating 1 μmol of *p*-nitrocatechol/min under the above assay conditions.

E. tenella arylsulphatase was assayed by measuring the production of *p*-nitrocatechol from *p*-nitrocatechol sulphate. The assay mixture consisted of 200 mM-sodium acetate buffer, pH 4.3, 8 mM-*p*-nitrocatechol sulphate and up to 100 μg of protein in a total volume of 0.5 ml. The

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mixture was incubated for 30 min at 37 °C and the reaction was stopped by the addition of 2.5 ml of 0.5 M-NaOH. The amount of *p*-nitrocatechol formed was determined as described above.

Purification of chicken caecal arylsulphatase

Groups of 50 White Leghorn chickens were obtained when 1 day old and maintained in a coccidia-free environment. When 3 weeks old, chickens were killed and caecae (45–50 g) were homogenized in 200 ml of 0.05 M-Tris/HCl buffer, pH 7.4, containing 0.25 M-NaCl in a Waring blender for 2 min. The homogenate was centrifuged at 20000 *g* for 30 min, and the supernatant was fractionated with (NH₄)₂SO₄.

(NH₄)₂SO₄ fractionation. The supernatant was made 40% saturated by the addition of solid (NH₄)₂SO₄ with constant mechanical stirring. After equilibration for 1 h, the solution was centrifuged at 27000 *g* for 30 min in a Sorvall centrifuge with a SS-34 rotor. The supernatant was discarded and the pellet was dissolved in 15 ml of cold water and dialysed against water until sulphate-free. Finally the solution was dialysed against 0.02 M-Tris/HCl buffer, pH 7.4, containing 1 M-NaCl, 1 mM-CaCl₂ and 1 mM-MnCl₂.

Concanavalin A-Sepharose chromatography. The dialysed solution (20 ml) was then applied to a column (2 cm × 15 cm) of concanavalin A-Sepharose equilibrated in 0.02 M-Tris/HCl buffer, pH 7.4, containing 1 M-NaCl, 1 mM-CaCl₂ and 1 mM-MnCl₂, at room temperature (Farooqui, 1976a; Farooqui & Srivastava, 1979). The column was washed with 5 ml portions of the same buffer at a flow rate of 0.15 ml/min. After 24 fractions had been collected, the column was eluted with a gradient formed from 125 ml of the starting buffer + 125 ml of 0.5 M-methyl α-D-mannoside in the same buffer. The active fractions were pooled and concentrated to 10 ml by ultrafiltration. This solution was dialysed against 0.05 M-Tris/HCl buffer, pH 7.4, and the resultant solution was centrifuged at 27000 *g* for 30 min. The pellet was discarded and the supernatant was applied to a cyclic AMP-Sepharose column.

Cyclic AMP-Sepharose chromatography. The above supernatant was loaded on a column (1.0 cm × 15 cm) of cyclic AMP-Sepharose equilibrated in 0.05 M-Tris/HCl buffer, pH 7.4. The column was washed with the same buffer at a flow rate of 0.2 ml/min and 2.5 ml fractions were collected.

After 100 ml of 0.05 M-Tris/HCl buffer, pH 7.4, had

been collected, the elution was continued with 2.0 mM-cyclic AMP in the same buffer. Active fractions were pooled, concentrated to 2.5 ml by ultrafiltration, and dialysed against 0.05 M-Tris/HCl buffer, pH 7.4.

Isolation of oocysts and sporozoites of *E. tenella*

Groups of 50 White Leghorn chickens were obtained when 1 day old and maintained in a coccidia-free environment. When 3 weeks old, each chicken was infected with 5 × 10⁵ sporulated oocysts of *E. tenella* (WIS); 7 days after infection, the chickens were killed. Caecal cores (100–150 g) were removed and homogenized with 600 ml of water for 1.5 min in a Waring blender. The homogenate was centrifuged at 1000 *g* for 10 min in a Sorvall centrifuge. The supernatant was discarded and the pellet was washed three times by suspension in distilled water and subsequent centrifugation at 800 *g* for 10 min. The pellet obtained was resuspended with 800 ml of distilled water in a Waring blender for 1 min and adjusted to pH 8.0 with 1 M-NaOH. Trypsin (0.25%) was added with constant mechanical stirring, and the preparation was kept at room temperature for 1 h. After addition of trypsin, the pH of the solution was maintained at 8.0 by addition of 1 M-NaOH. The trypsin-treated suspension was filtered through cheese-cloth. The filtrate containing the oocysts was adjusted to 12.5% (w/v) NaCl by addition of solid NaCl. The floating oocysts were collected by centrifugation at 1000 *g* for 5 min in a Sorvall RC2B refrigerated centrifuge equipped with a HSA rotor. The oocysts were washed twice with distilled water and suspended in 2% (w/v) potassium dichromate. This suspension was aerated for 3 days in a graduated cylinder (Farooqui & Hanson, 1983). The resultant sporulated oocysts were washed several times with water to remove potassium dichromate. Finally the oocyst pellet was treated with 10% Chlorox (bleach) for 15 min, and then washed with water until the Chlorox was completely removed. The purified oocysts were concentrated by centrifugation at 1000 *g* for 10 min and suspended in 0.02 M-Tris/HCl buffer, pH 7.4. Numbers of oocysts per given volume were determined by the procedure described by Roberson *et al.* (1973). Excystation of sporocysts was done by the method of Patton & Brigman (1979), and sporozoites were isolated by the method of Wagenbach (1969), with a glass-bead column (2.5 cm × 16 cm). Sporozoites were suspended in 0.02 M-Tris/HCl buffer, pH 7.4, and sonicated for two periods of 2 min at 5 min intervals at 0 °C with a sonic Dismembrator model 300 (Fisher) operating at full power (Farooqui *et al.*, 1983). The sonicated suspension was centrifuged at 12000 *g* for 30 min at 4 °C, and the

Table 1. Purification of arylsulphatase from chicken caecum

One unit of enzyme activity was defined as the amount liberating 1 μmol of *p*-nitrocatechol/min under the conditions described in the text.

Fraction	Total protein (mg)	Total activity (units)	Sp. activity (units/mg)	Yield (%)
Homogenate	3000	81	0.027	100
20000 <i>g</i> supernatant	600	60	0.100	74
0–40% -satd.-(NH ₄) ₂ SO ₄	200	50	0.250	62
Concanavalin A-Sepharose	15	44	2.93	54
Cyclic AMP-Sepharose	2.0	38	19.00	47

Fig. 1. Elution profile of chicken caecal arylsulphatase through a concanavalin A-Sepharose column

The experimental conditions and analysis of fractions for protein and enzyme activity are described in the Materials and methods section: —, A_{280} ; ----, arylsulphatase activity. Methyl α -D-mannoside gradient was applied at the arrow.

resultant supernatant was fractionated by the addition of solid $(\text{NH}_4)_2\text{SO}_4$ (0–40% saturation). The pellet containing most enzymic activity was dialysed overnight against 0.05 M-Tris/HCl buffer, pH 7.4, at 4 °C.

Lectin chromatography of *E. tenella* arylsulphatase

Concanavalin A-Sepharose chromatography was carried out as described above. *Ricinus communis* lectin-Sepharose 4B chromatography was done under the conditions of Surolia *et al.* (1975).

Determination of M_r

The M_r of arylsulphatases was determined by gel filtration (Andrews, 1965) on Sephacryl G-200 (2 cm $\times$ 45 cm). The marker proteins were catalase (M_r 232000), bovine serum albumin (M_r 66100), ovalbumin (M_r 43000), chymotrypsinogen A (M_r 25000) and ribonuclease (M_r 13700).

Protein was determined by the method of Bradford (1978), with bovine serum albumin as the standard. The specific activity was expressed as μmol of product formed/min per mg of protein.

Polyacrylamide-gel electrophoresis

This was carried out at room temperature by the method of Brewer & Ashworth (1969), with 7.5%-acrylamide gels in 0.03 M-Tris/glycine buffer, pH 8.4, for 2 h at a current of 3 mA/tube. Bromophenol Blue was used as a tracking dye. For localization of enzyme activity, unstained gels were cut into 1 cm segments and incubated in the reaction mixture for 2–3 h. Enzyme activity was determined as described above. Periodic acid-Schiff staining for glycoprotein was done by the method of Segrest & Jackson (1972).

RESULTS

Chicken caecal arylsulphatase was purified 700-fold from the homogenate by $(\text{NH}_4)_2\text{SO}_4$ fractionation and concanavalin A- and cyclic AMP-Sepharose chromatographies (Table 1). In four preparations made by the above procedure the mean yield was 45%. The elution profile of chicken caecal arylsulphatase through concana-

Fig. 2. Elution profile of chicken caecal arylsulphatase through a cyclic AMP-Sepharose column

The experimental conditions and analysis of fractions for protein and enzyme activity are described in the Materials and methods section: ●, A_{280} ; ○, arylsulphatase activity (A_{515}). Cyclic AMP (2.0 mM) was applied at the arrow.

valin A-Sepharose is shown in Fig. 1. Approx. 85–90% of protein was washed out from the column, and the enzyme was completely retained. The enzyme appeared as a sharp peak when the column was eluted with a linear gradient of methyl α -D-mannoside.

The elution profile of chicken caecal arylsulphatase A through cyclic AMP-Sepharose is shown in Fig. 2; about 85% protein was washed out from the column and the enzyme was completely retained. The enzyme was eluted as a sharp peak when the column was washed with 2.0 mM-cyclic AMP. The purity of the final enzyme preparation was evaluated by polyacrylamide-gel electrophoresis (Helwig *et al.*, 1977), which indicated that the preparation was not homogeneous; it showed at least four bands. Determination of arylsulphatase activity in 1 cm segments of the gel after elution indicated that the enzyme activity was located in the major protein band. Staining of an intact polyacrylamide gel indicated that the enzyme activity band was periodate-Schiff-positive.

Purification of *E. tenella* arylsulphatase is shown in Table 2. This procedure gave 28-fold purification of this enzyme, with an overall recovery of 71%. *E. tenella* arylsulphatase did not interact with concanavalin A-Sepharose and cyclic AMP-Sepharose under the conditions described for chicken caecal enzyme. Further, arylsulphatase from *E. tenella* did not interact with *Ricinus communis* lectin-Sepharose under the conditions described by Surolia *et al.* (1975). Staining of intact gel after electrophoresis (Segreat & Jackson, 1972) indicated that the enzyme activity band was not periodate-Schiff-positive.

The kinetic parameters of chicken caecal arylsulphatase and *E. tenella* arylsulphatase are shown in Table 3. Chicken caecal arylsulphatase showed optimal activity at pH 5.5, and the *E. tenella* enzyme at 4.3. The activity/time curve (Fig. 3) of *E. tenella* arylsulphatase indicated that the hydrolysis of *p*-nitrocatechol sulphate was proportional to time, at least up to 90 min. In contrast, chicken caecal arylsulphatase showed a linear rate for 15 min (Fig. 3), and then the activity/time response became anomalous (Baum & Dodgson, 1958). The chicken caecal enzyme showed more affinity for *p*-nitrocatechol sulphate (K_m 0.3 mM) than did the *E. tenella* arylsulphatase (K_m 2.5 mM). Na_2SO_4 and Na_3PO_4 inhibited chicken caecal arylsulphatase in a

Table 2. Purification of arylsulphatase from sporozoites of *E. tenella*

One unit of enzyme activity was defined as the amount liberating 1 μmol of *p*-nitrocatechol/min under the conditions described in the text.

Fraction	Total protein (mg)	Total activity (units)	Sp. activity (units/mg)	Yield (%)
Sonicated sporozoites	180	14	0.07	100
12000 <i>g</i> supernatant	20	12	0.60	86
0–40% -satd.-(NH ₄) ₂ SO ₄	5	10	2.0	71

Table 3. Kinetic parameters of chicken caecal and *E. tenella* arylsulphatases

Results are means $\pm$ S.D. for five determinations.

Parameter	Substrate . . .	Chicken		<i>E. tenella</i>
		<i>p</i> -Nitrocatechol sulphate	Cerebroside 3-sulphate	<i>p</i> -Nitrocatechol sulphate
pH optimum		5.5	4.8	4.3
K_m (mM)		0.3±0.05	0.13±0.005	2.5±0.13
V_{max} . (μ mol/min per mg)		20.0±0.8	3.5±0.15	2.0±0.05
K_i for sulphate (μ M)		50.0±5	15±2	300±35
K_i for phosphate (μ M)		20.0±4	8±0.5	90±15

competitive manner, with K_i values of 50 μM and 20 μM respectively (Table 3). For *E. tenella* arylsulphatase, SO_4^{2-} and PO_4^{3-} ions had K_i values of 300 μM and 90 μM respectively. Chicken caecal arylsulphatase hydrolysed cerebroside 3-sulphate optimally at pH 4.8, with an apparent K_m value of 0.13 mM. Cerebroside 3-sulphate is not hydrolysed by *E. tenella* arylsulphatase. Substrate specificity of chicken caecal arylsulphatase is shown in Table 4. It hydrolyses *p*-nitrocatechol sulphate, cerebroside 3-sulphate and ascorbic acid 2-sulphate. The rate of hydrolysis of *p*-nitrocatechol sulphate was highest, followed by ascorbic acid 2-sulphate and cerebroside 3-sulphate. *E. tenella* arylsulphatase hydrolysed ascorbic acid 2-sulphate at a slower rate (5 times less) than did the chicken caecal arylsulphatase. Chicken caecal enzyme hydrolysed *p*-nitrophenol sulphate at a rate similar to that for *p*-nitrocatechol sulphate. Cyclic AMP (5 and 10 mM) produced 25% and 47% inhibition of chicken caecal arylsulphatase respectively, but had no effect on the *E. tenella* enzyme. This explains the retention of chicken caecal arylsulphatase on a cyclic AMP-Sepharose column and its elution by cyclic AMP. It should be noted here that enzyme fractions from the cyclic AMP-Sepharose column were not very active, but after dialysis against 0.02 M-Tris/HCl buffer, pH 7.4, they became fully active. The latter step resulted in removal of cyclic AMP.

The effect of glycosaminoglycans on chicken caecal and *E. tenella* arylsulphatases is shown in Table 5. Glycosaminoglycans had no effect on chicken caecal arylsulphatase, whereas *E. tenella* arylsulphatase was strongly inhibited by chondroitin sulphate and heparin. Heparin was a more potent inhibitor than chondroitin sulphate. Thus, at 2 mg, heparin and chondroitin sulphate produced 80% and 35% inhibition of *E. tenella* arylsulphatase respectively. Hyaluronic acid, which does

Fig. 3. Plots of activity versus time for chicken caecal and *E. tenella* arylsulphatases

Composition of reaction mixture and assay conditions are described in the Materials and methods section. Arylsulphatase: ----, chicken caecal; —, *E. tenella*.

not contain a sulphate group, had no effect on the *E. tenella* enzyme. The M_r of *E. tenella* arylsulphatase was 49000. Chicken caecal arylsulphatase had M_r 97000 at pH 5.0 and 7.4. Chicken caecal enzyme interacted with DEAE-cellulose and was retained on this column (results not shown). Preparations of *E. tenella* arylsulphatase did not interact with DEAE-cellulose under a variety of conditions of ionic strength and pH.

The purified chicken caecal arylsulphatase can be stored at -20°C for 2 months without any appreciable loss of enzymic activity. *E. tenella* arylsulphatase, on the other hand, was quite unstable; it lost 60% of its activity

Table 4. Substrate specificity of chicken caecal and *E. tenella* arylsulphatases

The activity towards cerebroside 3-sulphate and ascorbic acid 2-sulphate was determined by the method of Waheed & Van Etten (1978) and with UDP-galactosamine 4-sulphate by the method of Fluharty *et al.* (1975). Results are means $\pm$ S.D. for three determinations.

Substrate	Arylsulphatase activity (μ mol/min per mg of protein)	
	Chicken caecum	<i>E. tenella</i>
<i>p</i> -Nitrocatechol sulphate	20.80 $\pm$ 4.0	2.0 $\pm$ 0.3
<i>p</i> -Nitrophenyl sulphate	15.5 $\pm$ 3.0	1.8 $\pm$ 0.2
Ascorbic acid 2-sulphate	8.8 $\pm$ 1.2	1.7 $\pm$ 0.3
Cerebroside 3-sulphate	3.5 $\pm$ 0.15	0.0
UDP- <i>N</i> -acetylgalactosamine 4-sulphate	0.0	0.0

in 2 days at -20°C . Attempts to stabilize this enzyme by the addition of sucrose and glycerol were not successful.

DISCUSSION

The present paper is the first report on purification and characterization of arylsulphatases from the chicken caecum (host tissue) and sporozoites of *E. tenella* (parasite). The retention of chicken caecal arylsulphatase on a concanavalin A-Sepharose column and its elution by methyl α -D-mannoside indicated the glycoprotein nature of this enzyme. A similar elution profile was obtained for arylsulphatase of chicken brain (Bishayee *et al.*, 1971; Ahmad *et al.*, 1977). Arylsulphatases A and B from various mammalian sources have been reported to be glycoproteins (Graham & Roy, 1973; Balasubramanian & Bachhawat, 1975; Farooqui & Roy, 1976; Farooqui, 1976a, 1981). In contrast, arylsulphatase from *E. tenella* is not retained on concanavalin A-Sepharose. This suggests that the *E. tenella* enzyme does not contain mannose and glucose residues. Further, *E. tenella* arylsulphatase does not interact with *Ricinus communis* lectin-Sepharose, indicating the absence of galactose residues. Chicken caecal arylsulphatase gave a positive result on periodate/Schiff staining for glycoprotein, whereas *E. tenella* arylsulphatase gave a negative result. These observations suggest the *E. tenella* arylsulphatase is not a glycoprotein. Chicken caecal arylsulphatase A is inhibited by cyclic AMP and is absorbed by a cyclic AMP-Sepharose column. A similar observation was made by Uchida *et al.* (1981) for ox liver arylsulphatase A. In contrast, *E. tenella* arylsulphatase is not affected by cyclic AMP (up to 20 mM). Arylsulphatases from other sources (O'Fagain *et al.*, 1980; Farooqui & Srivastava, 1981) were inhibited by nucleoside phosphates.

Like arylsulphatases A of mammalian (Roy, 1953) and avian (Farooqui & Bachhawat, 1971, 1972) origins, chicken caecal enzyme showed an abnormal anomalous time relationship (Baum & Dodgson, 1958). In contrast,

Table 5. Effect of glycosaminoglycans on chicken caecal and *E. tenella* arylsulphatases

Purified arylsulphatases were kept with glycosaminoglycan at 4°C for 30 min in 200 mM-sodium acetate buffer, pH 5.5 (chicken caecum enzyme) and pH 4.3 (*E. tenella* enzyme), with occasional shaking. The reaction was started by the addition of *p*-nitrocatechol sulphate (4 mM for caecal enzyme and 8 mM for *E. tenella* enzyme). The reaction was stopped by the addition of 0.5 M-NaOH as described in the text. A control reaction mixture containing all the components except glycosaminoglycan was treated in the same way. Results are means $\pm$ S.D. for three determinations.

Glycosaminoglycan	Concn. (mg)	Arylsulphatase activity (μ mol/min per mg of protein)	
		Chicken caecum	<i>E. tenella</i>
Control	—	17.5 $\pm$ 0.2	2.0 $\pm$ 0.3
Hyaluronic acid	0.5	18.0 $\pm$ 0.3	2.1 $\pm$ 0.3
	1.0	18.0 $\pm$ 0.2	2.0 $\pm$ 0.2
	2.0	18.3 $\pm$ 0.2	2.0 $\pm$ 0.3
Chondroitin sulphate	0.5	17.6 $\pm$ 0.2	1.8 $\pm$ 0.05
	1.0	17.9 $\pm$ 0.2	1.5 $\pm$ 0.05
	2.0	18.0 $\pm$ 0.2	1.3 $\pm$ 0.05
Heparin	0.5	17.5 $\pm$ 0.3	1.2 $\pm$ 0.03
	1.0	17.5 $\pm$ 0.3	0.8 $\pm$ 0.05
	2.0	18.5 $\pm$ 0.2	0.4 $\pm$ 0.04

arylsulphatase from *E. tenella* had a linear activity/time response up to 90 min. *E. tenella* arylsulphatase differs from chicken caecal enzyme not only in its kinetic parameters for *p*-nitrocatechol sulphate but also in its substrate specificity. The rate of hydrolysis of ascorbic acid 2-sulphate is 5 times slower than with chicken caecal arylsulphatase. Further, chicken caecal enzyme hydrolyses cerebroside 3-sulphate at a rate similar to that with chicken brain arylsulphatase A (Farooqui & Bachhawat, 1972, 1973; Ahmad *et al.*, 1977) and arylsulphatases A of other sources (Porter *et al.*, 1972). *E. tenella* arylsulphatase does not hydrolyse cerebroside 3-sulphate. Both arylsulphatases are strongly inhibited by sulphate and phosphate ions in a competitive manner. This property is similar to arylsulphatases of avian (Farooqui & Bachhawat, 1971, 1972) and mammalian origin (Roy, 1976; Dodgson & Rose, 1975). The K_i values for sulphate and phosphate were 50 μM and 20 μM respectively. Very different K_i values (300 μM and 90 μM respectively) were obtained for the *E. tenella* enzyme.

Chicken caecal and *E. tenella* arylsulphatases can also be distinguished on the basis of inhibition by heparin. This glycosaminoglycan had no effect on chicken caecal arylsulphatase, whereas *E. tenella* enzyme was very strongly inhibited. It should be noted here that the unique structure and surface charge distribution of heparin allow it to interact strongly with lysosomal hydrolases, enzymes of nucleic acid metabolism, tyrosine hydroxylases and lipases (Farooqui & Horrocks, 1984). These interactions either increase or decrease the biological activities of the above enzymes. However, the significance of heparin-enzyme interactions is not fully understood (Farooqui & Horrocks, 1984). The inhibition

of *E. tenella* arylsulphatase by heparin is a unique property which is not shared by any arylsulphatase activity of animal or plant origin (Farooqui, 1987).

Similarly to chicken brain arylsulphatase A, chicken caecal arylsulphatase had an M_r of 97000. Preliminary studies have indicated that it was made up of subunits of M_r about 50000. *E. tenella* arylsulphatase, on the other hand, had an M_r of 49000 and did not break into subunits on SDS/polyacrylamide-gel electrophoresis.

In conclusion, our studies have indicated that chicken caecal arylsulphatase can be purified by chromatography on concanavalin A- and cyclic AMP-Sepharose. This arylsulphatase resembles arylsulphatases A of other avian (Farooqui & Bachhawat, 1971, 1972; Ahmad *et al.*, 1977; Fedacka-Bruner & Croisille, 1978) and mammalian (Roy, 1976; Dodgson & Rose, 1975; Farooqui, 1980; 1981, 1987) tissues in its response to inhibition by sulphate and phosphate ions, its M_r and its glycoprotein nature. Further, like arylsulphatases A of other sources, chicken caecal enzyme hydrolyses cerebroside 3-sulphate (Mehl & Jatzkewitz, 1968; Farooqui & Bachhawat, 1972, 1973; Porter *et al.*, 1972).

Chicken caecal enzyme markedly differed from *E. tenella* arylsulphatase in kinetic parameters, substrate specificity, response to inhibition by heparin, glycoprotein nature and M_r .

Preliminary studies have indicated that the incubation of purified chicken caecal arylsulphatase with cerebroside 3-sulphate extracted from caecal membranes results in a rapid desulphation of this sulpholipid. The V_{max} for this hydrolysis was 3.1 $\mu\text{mol}/\text{min}$ per mg of protein. The role of *E. tenella* arylsulphatase in sporozoites is not understood at present. However, it may be possible that this enzyme, along with other acid hydrolases and proteinases, could be involved in the degradation and penetration of the membrane structures of the epithelial cells of the chicken caecum (Farooqui *et al.*, 1983), or the action of parasitic hydrolases on the host cell surface may expose certain receptors which are necessary for the parasite-host interaction.

We are grateful to Dr. L. McDougald and Dr. P. Long of the University of Georgia for providing cultures of *Eimeria tenella* (WIS), and Dr. R. Lujan for supplying sporozoites of this parasite. The work was supported in part by grant no. 901-15-174 from the U.S. Department of Agriculture.

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