Purification and Some Properties of Arylsulphatases A and B from Rabbit Kidney Cortex

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Arylsulphatases A and B (EC 3.1.6.1) of rabbit kidney cortex were purified 5250- and 7720-fold respectively by a multiple-column-chromatography method. The specific activity toward 4-nitrocatechol sulphate was $42\ \mu\text{mol/min per mg}$ for arylsulphatase A and $62\ \mu\text{mol/min per mg}$ for arylsulphatase B. Each enzyme migrated as a single band on polyacrylamide-gel electrophoresis, and the enzyme activity corresponded to the band of protein on the gel. The rate of hydrolysis of ascorbic acid 2-sulphate by arylsulphatase A was three times that for cerebroside 3-sulphate. Arylsulphatase B hydrolysed UDP-*N*-acetylgalactosamine 4-sulphate and glucosamine 4,6-disulphate, but not galactosamine 6-sulphate.

Arylsulphatases (arylsulphate sulphonydrolase, EC 3.1.6.1), the group of enzymes catalysing the hydrolysis of arylsulphates, are widely distributed in various animals. Depending on substrate specificity, response to various inhibitors and subcellular localization, three arylsulphatases have been identified in various animal tissues. Arylsulphatases A and B occur in the lysosomes. They are strongly inhibited by SO_4^{2-} and PO_4^{2-} ions. Arylsulphatase C, on the other hand, occurs in the microsomal fraction. This enzyme is characterized by its extreme insolubility and insensitivity to inhibition by SO_4^{2-} and PO_4^{2-} ions (Dodgson & Spencer, 1957; Dodgson & Rose, 1970; Roy & Trudinger, 1970). Arylsulphatases A and B have been extensively purified from various animal tissues (Nichol & Roy, 1964; Allen & Roy, 1968; Bleszynski *et al.*, 1969; Farooqui & Bachhawat, 1972; Bleszynski & Roy, 1973; Lee & Van Etten, 1975; Stevens *et al.*, 1975). Arylsulphatase A is an acidic glycoprotein (Graham & Roy, 1973). It differs from the corresponding arylsulphatase B, which is a slightly basic glycoprotein (Allen & Roy, 1968; Farooqui & Roy, 1976), in many physicochemical and kinetic properties (Dodgson & Rose, 1970; Nicholls & Roy, 1971). Further, at least in ox tissues, rabbit cornea and human placenta, arylsulphatase B occurs in multiple forms (Wortman, 1962; Allen & Roy, 1968; Gniot-Szulzycka, 1972; Farooqui & Roy, 1976), which probably differ from each other in their contents of *N*-acetylneuraminic acid (Farooqui, 1976a).

The physiological role of arylsulphatases A and B has been established recently. It is reported that arylsulphatase A is a cerebroside 3-sulphatase and its

deficiency causes the disease metachromatic leucodystrophy, which is characterized by an abnormal accumulation of cerebroside 3-sulphate in various organs and by excessive excretion of this lipid in the urine (Mehl & Jatzkewitz, 1968; Moser, 1972; Farooqui & Bachhawat, 1973; Jerfy & Roy, 1973; Farooqui *et al.*, 1977). Arylsulphatase B is a *N*-acetylgalactosaminide 4-sulphatase (Fluharty *et al.*, 1975; Farooqui, 1976b; Farooqui & Roy, 1976) and its deficiency results in Maroteaux-Lamy syndrome (Stumpf *et al.*, 1973), a mucopolysaccharidosis characterized by a marked accumulation and excretion of dermatan sulphate. We have previously reported the distribution of arylsulphatases A and B in glomerular and tubular fractions of rabbit kidney cortex (Helwig *et al.*, 1977).

Several methods have been reported for the preparation of arylsulphatases A and B (Bleszynski & Roy, 1973; Lee & Van Etten, 1975; Stevens *et al.*, 1975; Farooqui & Roy, 1976; Agogbua & Wynn, 1976). Many of these methods have been developed by using animals that are difficult to maintain in the laboratory. The present paper describes a method for the preparation of 'homogeneous forms' of arylsulphatases A and B from rabbit kidney cortex.

Materials and Methods

Materials

4-Nitrocatechol sulphate (dipotassium salt), methyl α -D-mannoside and Coomassie Brilliant Blue were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Sephadex G-150 and G-200, DEAE-Sephadex A-50, CM-Sephadex C-50 and con-

canavalin A–Sephacrose were obtained from Pharmacia, Uppsala, Sweden. DEAE-cellulose DE-52 was purchased from Whatman (Reeve Angel, London E.C.4, U.K.). Acrylamide and *NN'*-methylenebisacrylamide were obtained from Eastman Organic Chemicals, Distillation Product Industries, Rochester, NY, U.S.A. All other chemicals used in the present study were of analytical grade.

Assay of arylsulphatases

The enzymes were assayed by measuring the production of nitrocatechol from 4-nitrocatechol sulphate. The reaction mixture consisted of 0.45 ml of 50 mM-sodium acetate buffer, pH 5.5, containing 12.5 mM-4-nitrocatechol sulphate and 0.05 ml of enzyme solution in a total volume of 0.5 ml. The tubes were incubated for 5 min at 37°C and the reaction was stopped by the addition of 2.4 ml of 0.4 M-NaOH. The amount of liberated nitrocatechol ($\epsilon_{510} = 12600 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$) was determined spectrophotometrically. One unit of enzyme activity was defined as the amount liberating 1 μmol of nitrocatechol/min under the above conditions. The specific activity was expressed as units per mg of protein.

Cerebroside 3-sulphate was prepared by the method of Farooqui & Bachhawat (1972) and the arylsulphatase A activity towards cerebroside 3-sulphate was determined by the method of Porter *et al.* (1972).

Ascorbic acid 2-sulphate was prepared by the method of Quadri *et al.* (1973). The barium salt of ascorbic acid 2-sulphate was converted into the potassium salt by passage through a column (0.5 cm $\times$ 8 cm) of Dowex 50 (X8; K⁺ form). The activity of arylsulphatase A was determined by the method of Fluharty *et al.* (1976).

UDP-*N*-acetylgalactosamine 4-sulphate was prepared by the method of Donovan *et al.* (1967). Glucosamine 4,6-disulphate and galactosamine 6-sulphate were synthesized by the method of Farooqui (1976b). Hexosamine sulphatase activity was determined as described by Farooqui (1976b) and the liberated sulphate was measured by the method of Dodgson (1961).

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. In highly purified fractions the protein was determined after precipitation with 10% (w/v) trichloroacetic acid.

Electrophoresis

Polyacrylamide-gel electrophoresis was carried out by the method of Davis (1962) in 0.3 M-Tris/HCl buffer, pH 8.9, containing 7.5% acrylamide and 0.14% *NNN'*-tetramethylethylenediamine, for 3 h, at 3.5 mA/gel. The proteins were stained by Coomassie Brilliant Blue [0.1% in 10% (v/v) acetic acid] for 2 h at

70°C. The gels were destained in methanol/acetic acid/water (4:1:15, by vol) for 24 h at 37°C.

Preparation of arylsulphatases A and B

All operations were carried out at 4°C unless otherwise mentioned. Kidney cortices (120 g) from 15 rabbits 'Fauves de Bourgogne' were homogenized six times in 5 mM-Tris/HCl buffer (pH 7.4)/20 mM-NaCl, in a VirTis apparatus for 2 min at intervals of 20 s (two kidney cortices in 30 ml). The cortical homogenate, after freezing and thawing five times, was centrifuged at 105000 g_{max} for 30 min in a Spinco model L ultracentrifuge (rotor R30). The pellet was discarded and the supernatant was adjusted to pH 4.8 with 0.5 M-sodium citrate buffer, pH 4.5. This solution was kept overnight at 4°C and centrifuged as above. The pellet was discarded and the supernatant was made 40% saturated by the addition of solid (NH₄)₂SO₄ with constant mechanical stirring. After 1 h, the solution was centrifuged at 5000 g_{max} for 1 h in an MSE model Mistral 6L centrifuge. The supernatant was discarded and the precipitate was dissolved in 500 ml of 0.1 M-Tris/HCl buffer (pH 7.4)/20 mM-NaCl and dialysed against 5 litres of 5 mM-Tris/HCl buffer (pH 7.4)/20 mM-NaCl, for 18 h. This preparation was called the final extract.

Sephadex G-150 gel filtration. The dialysed preparation was applied in five portions to the same column (5 cm $\times$ 80 cm) of Sephadex G-150 equilibrated with 5 mM-Tris/HCl buffer (pH 7.4)/20 mM-NaCl. The column was eluted in 15 ml fractions with the same buffer, at a flow rate of 1.25 ml/min (Fig. 1). The fractions that contained enzyme activity were pooled and made 40% saturated by the addition of solid (NH₄)₂SO₄ with constant mechanical stirring. After 1 h, the solution was centrifuged at 5000 g_{max} for

Fig. 1. Elution profile of rabbit kidney-cortex arylsulphatases through Sephadex G-150 column

The experimental conditions and the analysis of fractions for enzyme activity (—) and protein (---) are described in the Materials and Methods section; 15 ml fractions were collected.

1 h in an MSE centrifuge. The supernatant was discarded and the pellet was dissolved in 80 ml of 5 mM-Tris/HCl buffer, pH 7.4. This solution, after dialysis against 8 litres of the same buffer, was subjected to DEAE-cellulose column chromatography.

DEAE-cellulose chromatography. This solution was then applied to a column (2.6 cm × 16 cm) of DEAE-cellulose DE-52 equilibrated with 5 mM/HCl buffer, pH 7.4. The column was washed with 80 ml of this buffer, and then eluted with a linear gradient formed from 300 ml of 5 mM-Tris/HCl buffer, pH 7.4, and 300 ml of 0.25 M-NaCl in the same buffer; 4 ml fractions were collected, at a flow rate of 0.4 ml/min. The activity eluted with the NaCl gradient was arylsulphatase A, whereas the arylsulphatase that did not adsorb on the column was arylsulphatase B (Fig. 2). After this step arylsulphatases A and B were processed separately.

Arylsulphatase A. The active fractions of arylsulphatase A were pooled and concentrated to 20 ml by ultrafiltration on Diaflo UM-10 membrane with an Amicon apparatus (Amicon Corp., Lexington, MA, U.S.A.). The resultant solution was dialysed against 2 litres of 20 mM-Tris/HCl buffer (pH 7.6)/0.1 M-NaCl.

(a) **DEAE-Sephadex chromatography:** this fraction was chromatographed on a column (1.6 × 16 cm) of DEAE-Sephadex equilibrated in 20 mM-Tris/HCl buffer (pH 7.6)/0.1 M-NaCl. The column was washed with 80 ml of this buffer and then eluted with a linear gradient formed from 200 ml of this buffer and 200 ml of 0.25 M-NaCl in the same buffer (Fig. 3); 4 ml fractions were collected at a flow rate of 0.25 ml/min. The active fractions were pooled and dialysed against 20 mM-Tris/HCl buffer (pH 7.4)/150 mM-NaCl/1 mM-MnCl₂/1 mM-CaCl₂. The solution was then con-

Fig. 2. Elution profile of arylsulphatases A and B through a DEAE-cellulose column

The experimental details and the analysis of fractions for enzyme activity (—) and protein (----) are described in the Materials and Methods section. ····, NaCl gradient; 4 ml fractions were collected.

Fig. 3. Elution profile of rabbit kidney-cortex arylsulphatase A through a DEAE-Sephadex column

The experimental details and the analysis of fractions for enzyme activity (—) and protein (----) are described in the Materials and Methods section. ····, NaCl gradient; 4 ml fractions were collected.

centrated to 20ml by ultrafiltration with an Amicon cell (membrane UM-10).

(b) Concanavalin A-Sepharose chromatography: the above solution was then applied to a column (0.8cm×7cm) of concanavalin A-Sepharose equilibrated in 20mM-Tris/HCl buffer (pH7.4)/150mM-NaCl/1mM-MnCl₂/1mM-CaCl₂, at room temperature (20–25°C). The column was washed with the same buffer, collected in 2.5ml fractions at a flow rate of 0.25ml/min. After 10 fractions had been collected the column was eluted with 0.6M-methyl α -D-mannoside in the same buffer (Fig. 4). The active fractions were pooled and dialysed against 2 litres of 70mM-sodium acetate buffer (pH5.0)/30mM-NaCl for 18h. This dialysed fraction was concentrated to 6ml by a Minicon A-25 apparatus (Amicon) and then chromatographed on a column of Sephadex G-200.

Fig. 4. Elution profile of rabbit kidney-cortex arylsulphatase A through a concanavalin A-Sepharose column

The experimental conditions and the analysis of fractions for enzyme activity (—) and protein (----) are described in the Materials and Methods section. At the arrow, the activity was eluted with 0.6M-methyl α -D-mannoside; 2.5ml fractions were collected.

(c) Sephadex G-200 gel filtration: the enzyme fraction was chromatographed on a column (0.8cm×90cm) of Sephadex G-200 equilibrated in 70mM-sodium acetate buffer (pH5.0)/30mM-NaCl and eluted with the same buffer at a flow rate of 0.06ml/min; 2ml fractions were collected. The fractions that contained enzyme activity were pooled and concentrated to 1–2ml, by a Minicon BD15 apparatus, and dialysed against 0.1M-Tris/HCl buffer, pH7.4.

Arylsulphatase B. After DEAE-cellulose chromatography, fractions having arylsulphatase B activity were concentrated to 20ml by using UM-10 membrane in an Amicon ultrafiltration cell. This solution was dialysed against 2 litres of 0.05M-imidazole buffer, pH6.5.

CM-Sephadex chromatography: pH-gradient CM-Sephadex chromatography was carried out by the method of Allen & Roy (1968), on a 1.6cm×16cm column at a flow rate of 0.4ml/min. The column eluate was collected in 4ml fractions (Fig. 5). The active fractions were pooled and dialysed against 20mM-Tris/HCl buffer (pH7.4)/150mM-NaCl/1mM-MnCl₂/1mM-CaCl₂. This fraction was concentrated and applied to a column (0.8cm×7cm) of concanavalin A-Sepharose. The conditions of concanavalin A-Sepharose chromatography were identical with those described for arylsulphatase A.

Results

Arylsulphatases A and B of rabbit kidney cortex were simultaneously purified 5250- and 7720-fold respectively, with an overall recovery of 13%, by the procedure presented in this paper (Table 1). The purity of the final preparations of arylsulphatases A and B was judged by polyacrylamide-disc-gel electrophoresis at various pH values. Both the enzymes showed a single band when stained for protein (Fig. 6a). The homogeneous preparations of arylsulphatases A and B were mixed and subjected to co-

Fig. 5. CM-Sephadex elution profile of rabbit kidney-cortex arylsulphatase B

The experimental conditions and the analysis of fractions for enzyme activity (—) and protein (----) are described in the Materials and Methods section. ····, pH gradient; 4ml fractions were collected.

Table 1. *Summary of preparative scheme for rabbit kidney-cortex arylsulphatases A and B*

One unit of enzyme was defined as the amount of enzyme liberating 1 μ mol of nitrocatechol/min under the conditions described in the text.

Enzyme	Purification stage	Total activity (units)	Total protein (mg)	Specific activity (munits/mg)	Yield (%)
A+B	Homogenate	171	22256	8	100
	Final extract	156	4232	37	91.2
	Sephadex G-150	139	813	170	81.2
A	DEAE-Cellulose	106	131	801	62.2
	DEAE-Sephadex	76.5	30.5	2510	44.8
	Concanavalin A-Sephadex	45.1	2.0	23 100	26.4
	Sephadex G-200	17.2	0.4	42000	10.1
B	DEAE-Cellulose	13.5	133	101	7.9
	CM-Sephadex	9.0	5.4	1690	7.9
	Concanavalin A-Sephadex	6.4	0.1	61 800	3.7

Fig. 6. *Polyacrylamide-gel electrophoresis of purified preparations of arylsulphatases A and B*

Polyacrylamide gels in 0.5 cm $\times$ 4 cm tubes were used. The other conditions for electrophoresis are given in the text. (a) Coomassie Brilliant Blue staining of arylsulphatases A and B; (b) co-electrophoresis of purified arylsulphatases A and B. The gel was cut into 2 mm segments, which were analysed for enzyme activity.

electrophoresis. The gel was cut into 2 mm segments, and it was found that the enzyme activity corresponded to the protein band of each enzyme (Fig. 6b). It was not possible to separate arylsulphatases A and B by Sephadex G-150 chromatography. The total arylsulphatase activity appeared as a single symmetrical peak, which did not coincide with the main protein peak (see Fig. 1). The separation of aryl-

sulphatases A and B was achieved by DEAE-cellulose chromatography. The arylsulphatase activity eluted by the NaCl gradient was arylsulphatase A, whereas that which did not adsorb on to the column was arylsulphatase B (see Fig. 2). A similar observation was also made by Bleszynski (1967) and Harinath & Robins (1971) for the ox brain and human brain arylsulphatases.

DEAE-Sephadex chromatography (Fig. 3) of arylsulphatase A indicated that the enzyme was strongly retained in the column and it could only be eluted with a gradient of NaCl. The elution profile of rabbit kidney-cortex arylsulphatase A from a concanavalin A-Sephadex column (Fig. 4) showed that more than 93% of the protein was not retained, but the enzyme was completely retained in the column. The enzyme appears as a sharp symmetrical peak when the column was eluted with 0.6 M-methyl α -D-mannoside. The polyacrylamide-gel electrophoresis of the fraction from the concanavalin A-Sephadex column indicated that the enzyme preparation had three protein bands. The Sephadex G-200 chromatography of the fraction from the concanavalin A column at pH 5.0 resulted in complete separation of these contaminating proteins.

Arylsulphatase B was obtained as a homogeneous protein by CM-Sephadex and concanavalin A-Sephadex column chromatography. CM-Sephadex chromatography of arylsulphatase B, under the conditions of Allen & Roy (1968) showed a single peak of enzyme activity (Fig. 5). The concanavalin A chromatography elution profile of arylsulphatase B was identical with that obtained for arylsulphatase A.

Properties of arylsulphatases A and B of rabbit kidney cortex

Some kinetic properties of arylsulphatases A and B are shown in Table 2. In sodium acetate buffer,

Table 2. Some kinetic parameters of arylsulphatases A and B of rabbit kidney cortex with 4-nitrocatechol sulphate as a substrate

Kinetic parameter	Aryl-sulphatase A	Aryl-sulphatase B
pH optimum	5.5	6.0
K_m (mM)	0.8	2.0
V_{max} (μ mol/min per mg)	42.0	61.8

Fig. 7. Time courses of rabbit kidney-cortex arylsulphatases A and B

The usual assay conditions are described in the Materials and Methods section. 270 ng of arylsulphatase A and 6.4 ng of arylsulphatase B was used. ■, Arylsulphatase A under usual assay conditions; ▲, arylsulphatase A under conditions of Baum *et al.* (1959); ●, arylsulphatase B under usual assay conditions; ○, arylsulphatase B under conditions of Baum *et al.* (1959). Under the conditions of Baum *et al.* (1959) arylsulphatase A was determined in the presence of $\text{Na}_4\text{P}_2\text{O}_7$ and NaCl which are the appropriate inhibitors of arylsulphatase B. For arylsulphatase B, the assay system of Baum *et al.* (1969) requires a high substrate concentration and the presence of Ba^{2+} ions.

arylsulphatase A had a pH optimum at 5.5, whereas arylsulphatase B showed maximum activity at pH 6.0. The K_m values for arylsulphatases A and B were 0.8 and 2 mM respectively. These values are not different from those obtained for ox liver (Allen & Roy, 1968) and ox brain (Bleszynski *et al.*, 1969) arylsulphatases A and B. Balasubramanian & Bachhawat (1976) reported a surprisingly low K_m value for sheep brain arylsulphatase B with 4-nitrocatechol sulphate as substrate.

Arylsulphatase A of various sources showed abnormal kinetics (Roy, 1953; Baum & Dodgson, 1958; Roy & Trudinger, 1970; Farooqui & Bachhawat, 1971) when 4-nitrocatechol sulphate was used as substrate. The activity-versus-time curves for rabbit kidney-cortex arylsulphatases A and B are

shown in Fig. 7; this relationship for arylsulphatase A was anomalous under the conditions described in the text. The time course of the reaction under the conditions of Baum *et al.* (1959) was normal. The enzyme also showed anomalous kinetics with respect to protein concentration under the conditions described in the text. Like arylsulphatase B of other tissues (Nicholls & Roy, 1971), arylsulphatase B of rabbit kidney cortex showed a normal activity-versus-time and activity-versus-protein relationship. Balasubramanian & Bachhawat (1976) have indicated that their partially purified preparation of arylsulphatase B of sheep brain showed anomalous kinetics.

Specificity of arylsulphatases A and B of rabbit kidney cortex

The specificity of arylsulphatases A and B of rabbit kidney cortex was tested by various sulphate esters. Like pig kidney and ox liver arylsulphatase A (Mehl & Jatzkewitz, 1968; Jerfy & Roy, 1973), rabbit kidney-cortex arylsulphatase A hydrolysed cerebroside 3-sulphate at a rate of 110 μ mol/h per mg of protein (Table 3). The rate of hydrolysis of ascorbic acid 2-sulphate was over three times that with cerebroside 3-sulphate. Arylsulphatase B did not act on cerebroside 3-sulphate and ascorbic acid 2-sulphate under the conditions described by Porter *et al.* (1972), Roy (1975) and Fluharty *et al.* (1976). Like arylsulphatase B of ox liver (Farooqui, 1976b; Farooqui & Roy, 1976), arylsulphatase B of rabbit kidney cortex hydrolysed UDP-N-acetylgalactosamine 4-sulphate. The rate of hydrolysis of these hexosamine sulphates by kidney and liver enzymes was approximately the same. The arylsulphatases A and B of rabbit kidney cortex were completely inactive towards galactosamine 6-sulphate.

Table 3. Substrate specificity of arylsulphatases A and B of rabbit kidney cortex

The enzymes were assayed as described in the Materials and Methods section.

Substrate	Activity (μ mol/h per mg of protein)	
	Aryl-sulphatase A	Aryl-sulphatase B
Nitrocatechol sulphate	2520	3705
Cerebroside 3-sulphate	110	0
Ascorbic acid 2-sulphate	387	0
UDP-galactosamine 4-sulphate	0	63
Glucosamine 4,6-disulphate	0	41
Galactosamine 6-sulphate	0	0

Discussion

The purification procedure presented in this paper results in homogeneous preparations of arylsulphatases A and B in yields of 3.4 and 0.83 mg/kg of rabbit kidney cortex respectively. This is a much higher yield of purer material than was reported for ox liver arylsulphatases A and B (Nichol & Roy, 1964; Nicholls & Roy, 1971; Bleszynski & Roy, 1973; Farooqui & Roy, 1976).

Rabbit kidney-cortex arylsulphatase B is eluted as a single peak on CM-Sephadex column chromatography, carried out by the method of Allen & Roy (1968). This observation is of considerable interest in relation to earlier work. Allen & Roy (1968) and Farooqui & Roy (1976) observed two isoenzymes of arylsulphatase B ($B1\alpha$ and $B1\beta$) of ox liver. Bleszynski & Roy (1973) separated as many as seven isoenzymes of ox brain arylsulphatase B. The existence of three isoenzymes of arylsulphatase B was also reported in human placenta and human liver (Gniot-Szulzycka, 1972; Okada *et al.*, 1975). In contrast, Agobua & Wynn (1976), like us, have found the presence of one form of arylsulphatase B in human liver. So at this stage it still remains an open question whether the multiple forms (isoenzymes) of arylsulphatase B are of genetic origin and have a role in regulation of arylsulphatase B activity *in vivo* or whether they are artifacts arising during the purification procedure.

The retention of arylsulphatases A and B on a concanavalin A-Sepharose column and their elution by methyl α -D-mannoside indicates that the binding of these enzymes to concanavalin A-Sepharose is through the carbohydrate moiety. Similar observations were made by Bishayee *et al.* (1973) and Farooqui & Roy (1976) for sheep brain and ox liver arylsulphatases A and B. It should also be recalled here that Farooqui & Roy (1976) reported that the isoenzymes of arylsulphatase B can be partially separated by concanavalin A-Sepharose chromatography. In the present study concanavalin A-Sepharose chromatography of rabbit kidney-cortex arylsulphatase B did not indicate the presence of isoenzymes in this organ.

The kinetic properties of arylsulphatases A and B were similar to those reported for arylsulphatases A and B of other sources (Allen & Roy, 1968; Bleszynski *et al.*, 1969; Roy & Trudinger, 1970; Nicholls & Roy, 1971). Like arylsulphatase A of other sources, rabbit kidney-cortex arylsulphatase A can hydrolyse cerebroside 3-sulphate. The rate of hydrolysis was about one-twentieth of that for 4-nitrocatechol sulphate. Similar observations were made by various workers (Jerfy & Roy, 1973; Farooqui *et al.*, 1977). It could also hydrolyse ascorbic acid 2-sulphate, but at a much faster rate than cerebroside 3-sulphate. There was no release of

sulphate from cerebroside 3-sulphate and ascorbic acid 2-sulphate by arylsulphatase B of rabbit kidney cortex under the above conditions. Homogeneous preparations of arylsulphatase B of rabbit kidney cortex were able to hydrolyse UDP-N-acetyl-galactosamine 4-sulphate at a rate that was one-sixtieth of that for 4-nitrocatechol sulphate. In addition arylsulphatase B of rabbit kidney cortex can also hydrolyse glucosamine 4,6-disulphate, but did not act on galactosamine 6-sulphate. Arylsulphatase A could not hydrolyse these hexosamine sulphates under the conditions used by various workers (Fluharty *et al.*, 1975; Farooqui, 1976b; Farooqui & Roy, 1976).

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References

- Agobua, S. I. O. & Wynn, C. H. (1976) *Biochem. J.* **153**, 415–421
- Allen, E. & Roy, A. B. (1968) *Biochim. Biophys. Acta* **168**, 243–251
- Balasubramanian, K. & Bachhawat, B. K. (1976) *J. Neurochem.* **27**, 485–492
- Baum, H. & Dodgson, K. S. (1958) *Biochem. J.* **69**, 573–582
- Baum, H., Dodgson, K. S. & Spencer, B. (1959) *Clin. Chim. Acta* **41**, 453–455
- Bishayee, S., Farooqui, A. A. & Bachhawat, B. K. (1973) *Indian J. Biochem. Biophys.* **10**, 1–2
- Bleszynski, W. S. (1967) *Enzymologia* **32**, 169–181
- Bleszynski, W. S. & Roy, A. B. (1973) *Biochim. Biophys. Acta* **317**, 164–171
- Bleszynski, W. S., Leznicki, A. & Lewosz, J. (1969) *Enzymologia* **37**, 314–324
- Davis, B. J. (1962) preprint of *Disc Electrophoresis*, Distillation Products Division of Eastman Kodak Co., Rochester, NY
- Dodgson, K. S. (1961) *Biochem. J.* **78**, 312–319
- Dodgson, K. S. & Rose, F. A. (1970) in *Metabolic Conjugation and Metabolic Hydrolysis* (Williams, R. T. & Fishman, H. eds.), pp. 239–325, Academic Press, New York
- Dodgson, K. S. & Spencer, B. (1957) *Methods Biochem. Anal.* **4**, 211–255
- Donovan, J. W., Davis, J. G. & Park, L. U. (1967) *Arch. Biochem. Biophys.* **122**, 17–23
- Farooqui, A. A. (1976a) *Biochimie* **58**, 759–761
- Farooqui, A. A. (1976b) *Experientia* **32**, 1242–1244
- Farooqui, A. A. & Bachhawat, B. K. (1971) *J. Neurochem.* **18**, 635–646
- Farooqui, A. A. & Bachhawat, B. K. (1972) *Biochem. J.* **126**, 1025–1033
- Farooqui, A. A. & Bachhawat, B. K. (1973) *J. Neurochem.* **20**, 889–891
- Farooqui, A. A. & Roy, A. B. (1976) *Biochim. Biophys. Acta* **452**, 431–439

- Farooqui, A. A., Rebel, G. & Mandel, P. (1977) *Life Sci.* **20**, 569-584
- Fluharty, A. L., Stevens, R. L., Fung, D., Peak, S. & Kihara, H. (1975) *Biochem. Biophys. Res. Commun.* **64**, 955-962
- Fluharty, A. L., Stevens, R. L., Miller, R. T., Shapiro, S. S. & Kihara, H. (1976) *Biochim. Biophys. Acta* **429**, 508-516
- Gniot-Szulzycka, J. (1972) *Acta Biochim. Pol.* **19**, 181-190
- Graham, E. R. B. & Roy, A. B. (1973) *Biochim. Biophys. Acta* **329**, 88-92
- Harinath, B. C. & Robins, E. (1971) *J. Neurochem.* **18**, 245-257
- Helwig, J. J., Farooqui, A. A., Bollack, C. & Mandel, P. (1977) *Int. J. Biochem.* in the press
- Jerfy, A. & Roy, A. B. (1973) *Biochim. Biophys. Acta* **293**, 178-190
- Lee, G. D. & Van Etten, R. L. (1975) *Arch. Biochem. Biophys.* **166**, 280-294
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Mehl, E. & Jatzkewitz, H. (1968) *Biochim. Biophys. Acta* **151**, 619-627
- Moser, H. W. (1972) in *The Metabolic Basis of Inherited Diseases* (Stanbury, J. B., Wyngaarden, J. B. & Frederickson, D. S., eds.), pp. 688-729, McGraw-Hill, New York
- Nichol, L. W. & Roy, A. B. (1964) *J. Biochem. (Tokyo)* **55**, 643-651
- Nicholls, R. G. & Roy, A. B. (1971) *Enzymes 3rd Ed.* **5**, 21-41
- Okada, K., Collins, J., Worth, W. S. & Austin, J. (1975) *Biochem. Med.* **12**, 290-301
- Porter, M. T., Fluharty, A. L., Dela Flor, S. D. & Kihara, H. (1972) *Biochim. Biophys. Acta* **258**, 769-778
- Quadri, S. F., Seib, P. A. & Deyoe, C. W. (1973) *Carbohydr. Res.* **29**, 259-264
- Roy, A. B. (1953) *Biochem. J.* **55**, 653-661
- Roy, A. B. (1975) *Biochim. Biophys. Acta* **377**, 356-363
- Roy, A. B. & Trudinger, P. A. (1970) *The Biochemistry of Inorganic Compounds of Sulphur*, pp. 133-189, Cambridge University Press, Cambridge
- Stevens, R. L., Fluharty, A. L., Skokut, M. H. & Kihara, H. (1975) *J. Biol. Chem.* **250**, 2495-2501
- Stumpf, D. A., Austin, J. H., Crocker, A. C. & La France, M. (1973) *Am. J. Dis. Child.* **126**, 747-755
- Wortman, B. (1962) *Arch. Biochem. Biophys.* **97**, 70-74