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Comparative studies of murine anionic and cationic arylsulfatase B[1]

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Summary. Murine brain possesses an anionic form of arylsulfatase B which accounts for approximately 12–16% of non-microsomal arylsulfatase activity. This isozyme is antigenically similar to cationic arylsulfatase B, displays a similar developmental profile, and can be converted to a form resembling the cationic species by prior treatment with neuraminidase.

Arylsulfatases (arylsulfate sulfohydrolase, EC 3.1.6.1) occur in mammalian tissues in several forms which differ with respect to their intracellular distribution, substrate affinities, and physical and chemical properties[2]. Lysosomal arylsulfatase B appears to participate in the stepwise degradation of sulfated mucopolysaccharides by acting as an exosulfatase upon N-acetylgalactosamine-4-sulfate residues of chondroitin and dermatan sulfates[3]. Arylsulfatase B is deficient in the human mucopolysaccharidosis, Maroteaux-Lamy syndrome[4].

Multiple forms of arylsulfatase B have been detected in bovine, rabbit, and human tissues[5–7]. Stevens et al.[8] resolved human brain arylsulfatase B into major (cationic) and minor (anionic) species using DEAE-cellulose chromatography. Both species of enzyme exhibited similar substrate specificities and pH optima and were not inhibited by silver ion, an inhibitor of arylsulfatase A; however, the isozymes differed with respect to their apparent isoelectric points, electrophoretic mobilities, and thermolability.

We have detected both cationic and anionic forms of arylsulfatase B in murine tissue. This report describes the tissue distribution, physical and chemical properties, and developmental and immunological relationships of these isozymes.

Methods. Inbred mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, and raised to 45 days of age unless otherwise indicated. Arylsulfatase A and B activities were assayed using p-nitrocatechol-SO_4 as substrate[9].

Isolation of anionic arylsulfatase B. 50% w/v aqueous homogenates of C57BL/6J brain or liver were sonicated, centrifuged at 100,000× g for 60 min, and dialyzed overnight against 0.02 M Tris-acetate buffer, pH 7.4 (start buffer). 1 ml of retentate was applied to a 10×1.5 cm column of DEAE-cellulose preequilibrated with start buffer. Approximately 20 ml of start buffer were run through the column (flow rate: 12–13 ml/h) followed by 40 ml of a linear buffer-chloride gradient formed from 20 ml start buffer and 20 ml of 0.2 M Tris-acetate buffer, pH 7.4, containing 0.4 M NaCl, and 1-ml fractions were collected.

Electrofocusing of arylsulfatase isozymes. Arylsulfatase isozymes from the DEAE-cellulose peaks were electrofocused in narrow gradient (pH 5–8) ampholine-acrylamide slab gels[10]. The isoelectric points of the enzyme bands were estimated by pH measurements of extracts from parallel slices from the same gel. Enzyme bands were visualized by immersion in 10 mM p-nitrocatechol-SO_4 in acetate buffer, pH 5.9, at 37 °C for 30 min and in 1 N NaOH for 5 min.

Characterization of anionic arylsulfatase B. The Michaelis constants and pH optima of anionic and cationic arylsulfatase B and arylsulfatase A were determined by standard methods using p-nitrocatechol-SO_4 as substrate. The respective enzymes were preincubated at 37 °C for 15 min in the presence of 200 µM $AgNO_3$ prior to enzyme assay to measure silver inhibition. Immunological characterization of the 3 arylsulfatases was accomplished by mixing 400 µl of appropriately diluted DEAE-cellulose fraction with increasing quantities of rabbit anti-mouse kidney arylsulfatase B IgG, and the volume of the mixture was adjusted to 600 µl with 0.05 M imidazole-HCl buffer, pH 6.6, containing 0.1 M NaCl. The mixtures were incubated at 37 °C for 1 h and at 4 °C for 16 h, and the immunoprecipitate was pelleted at 6000× g. The effect of neuraminidase upon anionic arylsulfatase B was determined by the method of Farooqui and Srivastava[11]. The 100,000× g supernatant from the sonicated brain extract was incubated with type VI

Clostridium perfringens neuraminidase (Sigma Chemical Co.), with buffer, or with *C. perfringens* neuraminidase that had been heated at 100 °C for 10 min. The incubation mixtures were clarified by centrifugation, and the arylsulfatase isozymes of the supernatants were resolved by DEAE-cellulose chromatography. Heat inactivation of arylsulfatases from the DEAE-cellulose fractions was conducted in 0.5 M sodium acetate buffer, pH 5.9, containing 0.2 mg% (w/v) bovine serum albumin[9]. The isozymes were heated at 65 °C, and aliquots were withdrawn at 5–10 min intervals, cooled, and centrifuged. Residual enzyme activities of the supernatants were measured as described above.

Developmental variation of arylsulfatase activities. Arylsulfatase isozymes were resolved by DEAE-cellulose chromatography as previously described[9]. Male mice were sacrificed at 6-day intervals following birth, and pooled brain tissue from 3 mice was used as the source of the 3 isozymes.

Results and discussion. Brain arylsulfatase activity eluted in 3 peaks: cationic arylsulfatase B (fractions 5–12), anionic arylsulfatase B (arylsulfatase B′: fractions 21–25), and arylsulfatase A (fractions 33–38). Recoveries averaged 90–95% of the activity applied. Approximately 15% of the total arylsulfatase activity was recovered in the arylsulfatase B′ fraction. Liver arylsulfatase activity eluted from DEAE-cellulose in 2 peaks corresponding to arylsulfatases B and A respectively, indicating that arylsulfatase B′ accounts for less than 1% of liver arylsulfatase activity. The proportions and distributions of the 3 murine arylsulfatases resembled those reported by Stevens et al.[8] for the corresponding human enzymes.

Biochemical properties of arylsulfatases A, B, and B′ are presented in the table. Arylsulfatases B and B′ exhibited apparent Michaelis constants of 1.4 mM, had similar pH optima, and were not appreciably inhibited by 200 µM $AgNO_3$. These data suggest that arylsulfatase B′ may be structurally related to arylsulfatase B. This relationship was supported by the effect of neuraminidase digestion upon the elution profile of brain arylsulfatases from DEAE-cellulose. Digestion with neuraminidase resulted in a quantitative shift of activity from the arylsulfatase B′ peak to the arylsulfatase B fraction. No shift of activity occurred when brain arylsulfatases were incubated with neuraminidase that had been preheated at 100 °C. Activities recovered from the DEAE-cellulose columns programmed with untreated, neuraminidase-treated, or heated neuraminidase-treated arylsulfatases were comparable.

Arylsulfatases from each DEAE-cellulose peak focused in 2 or more bands in narrow gradient (pH 5–8) ampholine-acrylamide gels (fig. 1a). The arylsulfatase B′ fraction was resolved into 2 bands which were connected by a diffuse zone of activity stain. The apparent isoelectric points for the major bands or arylsulfatases A, B, and B′ were 4.6, 7.4, and 6.3, respectively. Pretreatment of 100,000 × g supernatant from sonicated brain extract with neuraminidase resulted in disappearance of enzyme from the arylsulfatase B′ region of the gel. These experiments strongly suggest that brain arylsulfatase B′ contains relatively more sialic acid compared to arylsulfatase B.

Further evidence for a structural relationship between arylsulfatases B and B′ is provided in the plots of supernatant activity vs. quantity of IgG presented in figure 1b. Both arylsulfatase B and B′ activities were precipitated by the IgG preparation, while arylsulfatase A activity was largely unaffected. These data strongly support structural similarity between the arylsulfatase B and B′ isozymes, since kidney contains negligible quantities of arylsulfatase B′, and the kidney arylsulfatase B used for immunization had been isolated from any contaminating arylsulfatase B′ during its 5000-fold purification[12].

The half-denaturation time (time required for one-half of

Biochemical properties of C57BL/6J brain arylsulfatases A, B, and B′

	Aryl A	Aryl B	Aryl B′
K_m (mM)	1.2	1.4	1.4
pH Optimum	5.3	5.9	5.9
pI	4.6	7.4	6.3
$t_{1/2}$ (65 °C; min)	6	35	14
Inhibition (%)			
200 µM $AgNO_3$	75	0	0

Aryl = arylsulfatase.

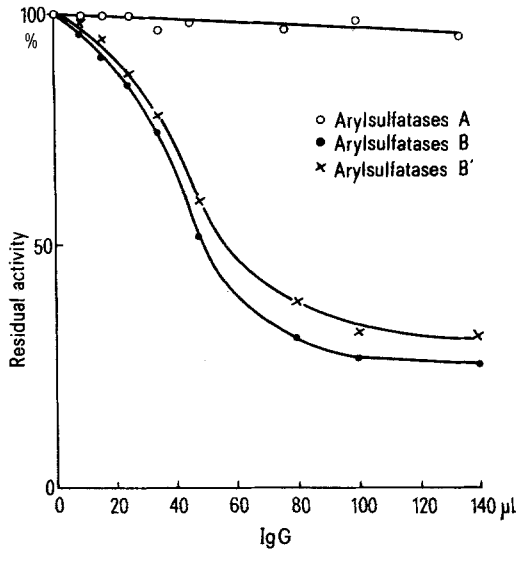

Figure 1. Brain arylsulfatases A, B, and B′. *a* Electrofocusing profiles of DEAE-cellulose fractions: arylsulfatase B′ (left); arylsulfatase A (center); and arylsulfatase B (right). *b* Immunoprecipitation of brain arylsulfatases with rabbit anti-murine kidney arylsulfatase B IgG.

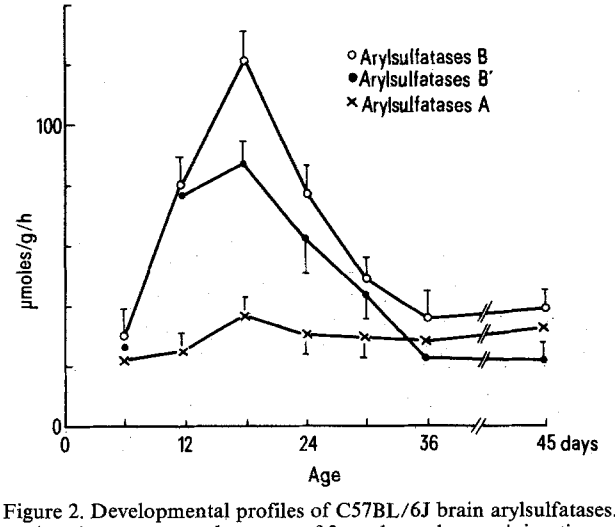

Figure 2. Developmental profiles of C57BL/6J brain arylsulfatases. Each point represents the mean of 3 pools, each containing tissues from 3 males. Error bars indicate the range for each age.

the initial enzyme activity to decay at 65 °C) for arylsulfatase B′ was about 14 min. Arylsulfatase B was more thermostable and arylsulfatase A more thermolabile than arylsulfatase B′ (table).

Both arylsulfatase B and B′ activities increase rapidly after 6 days of age and peak at approximately 18 days postnatal age (fig. 2), while developmental fluctuation of arylsulfatase A is less prominent. Similar developmental profiles were observed for SWR/J and A/J brain arylsulfatases. These trends suggest that arylsulfatases B and B′ may be subject to similar developmental regulation.

The immunotitration and neuraminidase experiments indicate that arylsulfatases B and B′ may be derived from the same primary gene product. SWR/J mice possess a more thermostable form of arylsulfatase B ($t_{1/2} \approx 75$ min) compared to C57BL/6J mice[13]. By contrast, SWR/J arylsulfatase B′ exhibited a half-denaturation time of 15 min which closely approximated that of C57BL/6J arylsulfatase B′. If arylsulfatases B and B′ are derived from the same primary gene product, then the posttranslational modification mechanism must sufficiently alter their structures to abolish their thermostability differences (with respect to arylsulfatase B′) or to enhance these differences (with respect to arylsulfatase B).

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Structural relationships between the endogenous volatile urinary metabolites of experimentally diabetic rats and certain neurotoxins[1]

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Summary. High resolution glass capillary gas chromatography and GC/MS were utilized to examine qualitative and quantitative variations from normal of urinary volatile metabolites of long-term alloxan and streptozotocin diabetic rats. Volatile metabolites were structurally compared with known neurotoxins to examine any possible relationship between these metabolites and the development of the diabetic polyneuropathy.

There is a growing body of evidence which implicates the axon as the initial site of damage in diabetic polyneuropathy in humans and experimental animals[3–8]. Strikingly similar axonal degeneration is seen in a number of other conditions. This includes many drug- and toxin-related polyneuropathies which exhibit a characteristic pattern of primary axonal nerve degeneration[9–12]. A recent report suggests the possibility that metabolic neuropathies such as diabetic polyneuropathy may arise from an abnormal in vivo production of neurotoxic metabolites[12].

Since previous reports[9–12] have implicated small organic molecules (e.g., n-hexane, 2,5-hexanedione, acrylamide and others) as potent axonal neurotoxic agents, we have investigated the possibility that such or chemically similar compounds are produced in vivo due to the diabetic condition. Urinary chromatographic profiles of volatile components were recorded for alloxan and streptozotocin diabetic rats. Volatile metabolites of physiological fluids suggest themselves for examination since these small neutral organic metabolites are chemically similar to known neurotoxic substances, and deviations in the concentration of some of these molecules from normal are seen in human diabetics[13,14] and alloxan diabetic rats shortly after the development of the hyperglycemic condition[15].

Based on the available evidence concerning the functional and structural changes in nerves that accompany the development of diabetic neuropathy in alloxan and streptozotocin diabetic rats[3,5–8], we have chosen experimentally diabetic animals of 2–12-month disease duration for this investigation. Comparisons with suitable control animals have enabled us to evaluate qualitative and quantitative variations in the volatile metabolites caused by the long-term diabetic condition. Metabolites exhibiting qualitative or large quantitative alterations from normal were structurally compared to known neurotoxins.

Materials and methods. Sprague-Dawley male rats (Harlan Industries, Indianapolis, Indiana) weighing between 150 and 170 g were utilized in these experiments. Streptozotocin diabetic rats received a single injection of 65 mg/kg citrated streptozotocin (a gift from the Upjohn Co., Kalamazoo, Michigan) in saline solution. Alloxan diabetic rats received a single injection, after a 24-h fast, of 40 mg/kg alloxan (alloxan monohydrate, Sigma Chemical Co., St. Louis, MO) in saline solution. All injections were i.v. using the tail vein of the rat. Solution concentrations were adjusted to keep injection volumes at approximately 1 ml. Control rats received a single injection of 1 ml saline solution.

Blood glucose values were measured with a Beckman Glucose Analyzer. Values were determined at 2-week inter-