Mammalian arylsulfatases A and B: relative rates of hydrolysis of artificial substrates

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Summary. Rodent and bovine arylsulfatase B hydrolyze 4-methylumbelliferyl sulfate (4MUS) 10- to 30-fold more efficiently than arylsulfatase A. Therefore, 4MUS grossly underestimates arylsulfatase A activity in the presence of excess arylsulfatase B.
Key words. Arylsulfatase A; arylsulfatase B; 4-methylumbelliferyl sulfate; rodent; bovine.

Arylsulfatases A and B (arylsulfate sulfohydrolase, EC 3.1.6.1) are lysosomal enzymes which hydrolyze sulfate ester bonds found in certain complex lipids (cerebroside 3-sulfate) and glycosaminoglycans (N-acetylgalactosamine 4-sulfate residues of chondroitin 4-sulfate and dermatan sulfate), respectively[2,3]. Both enzymes appear to play important roles in the turnover of these lipids and glycosaminoglycans in vivo, and deficiencies of arylsulfatases A and/or B are associated with a number of human lysosomal disorders[4–6]. Furthermore, these enzymes may be essential for normal fertilization[7].

The clinical importance of arylsulfatases has stimulated the development of relatively simple methods for their assay in body fluids and tissue extracts. Most of these assays employ artificial substrates such as p-nitrocatechol sulfate (pNCS) or 4-methylumbelliferyl sulfate (4MUS) in combination with sodium pyrophosphate or silver ion which preferentially inhibit arylsulfatase A[8,9]. The enzymes are typically assayed in the presence and absence of the inhibitor. Arylsulfatase B is believed to account for the residual activity in the presence of the inhibitor, and arylsulfatase A activity is obtained by difference. The validity of this approach rests upon the assumptions that 1) arylsulfatase B is unaffected by the inhibitor, and 2) that both isozymes have similar affinities for the substrate employed for the assay. We have found that bovine and rodent arylsulfatase A activities are underestimated when 4MUS is used as substrate. Furthermore, this substrate appears to be hydrolyzed by an enzyme having properties characteristic of arylsulfatase B.

Methods. SWR/J inbred mice and Sprague-Dawley rats were obtained from the Jackson Laboratory (Bar Harbor, ME) and Holtzman (Madison, WI), respectively. Adult liver was used as the source of enzyme. Beef liver was procured from local sources. pNCS-sulfatase activities were determined according to Harrison et al[10]. 4MUS-sulfatase activities were assayed by incubating 50 µl of appropriately diluted enzyme with 100 µl of 5 mM 4MUS in 0.5 M sodium acetate-acetic acid buffer (pH 5.1 for arylsulfatase A or pH 5.9 for arylsulfatase B) at 45 °C for 5 min (arylsulfatase A) or 10 min (arylsulfatase B). The reaction was stopped with 4 ml of 0.08 M glycine-sodium carbonate (pH 10), and fluorescence was measured with a Turner 111 fluorometer using 7–60 primary and 4–8 secondary filters. The 4MUS was repurified prior to use[11].

Anionic and cationic arylsulfatases were isolated using a multistep procedure. 10% (w/v) homogenates of rat, murine, or bovine liver were prepared in 10 mM Tris/HCl buffer (pH 7.5) containing 0.05% (v/v) Triton X-100, sonicated 30 sec, and centrifuged at 20,000 × g for 30 min. The supernatant was adjusted to pH 7.0 with 2 M Tris, and solid ammonium sulfate was added to 60% saturation. The slurry was allowed to stand overnight at 4 °C and was centrifuged at 3,000 × g for 15 min. The pellet was resuspended in 1/4 volume of 0.1 M Tris/HCl (pH 7.5)/0.1 M NaCl/0.05% (v/v) Triton X-100 and dialyzed overnight at 4 °C against 0.02 M Tris-acetic acid buffer (pH 7.4). Anionic and cationic arylsulfatases were separated by DEAE-Sephacel (Pharmacia) chromatography. The retentate was applied to a 2.5 × 40 cm column and washed with two column volumes of 0.02 M Tris-acetic acid buffer (pH 7.4), and the column was developed with a linear gradient formed from equal volumes of 0.02 M Tris-acetate buffer and 0.2 M Tris-acetic acid buffer (pH 7.4) containing 0.4 M NaCl. Arylsulfatases eluting from the column under high salt conditions were dialyzed against 0.02 M Tris/HCl (pH 7.2), concentrated by pressure filtration to 5–7 ml, and applied to a 2.5 × 50 cm column of Sephacryl S-300 (Pharmacia). The column was developed with 10 mM Tris/HCl (pH 7.2) containing 0.5 M NaCl. Enzyme fractions were located using the pNCS-sulfatase assay at pH 5.5. All enzyme fractions were dialyzed against 0.02 M Tris/HCl (pH 7.2), concentrated to 5 ml, and aliquoted for storage at − 80 °C until characterization.

Enzyme characterization. Optimal pH, Michaelis constants, linearity, electrophoretic mobility, isoelectric point, thermostability, and immunological characteristics of the arylsulfatase fractions were determined as previously described[12,13]. Effects of silver upon arylsulfatase activity were estimated by preincubation of the enzyme with 0.2 M silver nitrate at 37 °C for 15 min. The remaining inhibitors were incorporated into the reaction mixture at the indicated concentrations.

Results and discussion. Rodent and bovine pNCS-sulfatase activities were resolved into the expected anionic and cationic fractions by DEAE-Sephacel chromatography (table 1). The cationic isozyme (arylsulfatase B) predominated in rodent liver, while the anionic isozyme accounted for approximately 80% of the pNCS-sulfatase activity recovered from bovine liver. Rodent and bovine anionic pNCS-sulfatase activities eluted from Sephacryl S-300 at a point corresponding to a relative molecular weight of 110 ± 5 kD, the reported molecular weight for mammalian arylsulfatase A.

The rodent and bovine arylsulfatase B fractions contained a much larger proportion of 4MUS-sulfatase activity than the respective arylsulfatase A fractions, suggesting that 4MUS is inefficient for estimation of arylsulfatase A activity in the presence of arylsulfatase B. Furthermore, the pNCS-sulfatase/4MUS-sulfatase activity ratios of the rat and mouse anionic arylsulfatase fractions were increased 5-fold following Sephacryl S-300 gel filtration, while the corresponding ratio for the bovine enzyme fraction was unchanged by this procedure. We have previously demonstrated that a majority of rodent anionic 4MUS-sulfatase activity is associated with a 55 kD protein possessing properties characteristic for arylsulfatase B[14]. This 55 kD arylsulfatase B isozyme contributed little pNCS-sulfatase activity to the rodent anionic fraction and escaped detection when pNCS was used to monitor enzyme activity eluting from the Sephacryl S-300 column. The data in table 1 indicate that the 55 kD isozyme does not contribute appreciably to bovine anionic arylsulfatase activity.

Several properties of rodent anionic 4MUS-sulfatase activity more closely resembled those of arylsulfatase B than arylsulfatase A (tables 2 and 3). By contrast, rodent anionic pNCS-sulfa-

Table 1. Isolation of bovine and rodent hepatic anionic arylsulfatases

	Rat		Mouse		Bovine	
	pNCS*	R**	pNCS	R	pNCS	R
Crude supernatant	3436	14	2365	7	1592	6
Ammonium sulfate	3270	12	1870	6	1168	5
DEAE-Sephacel						
(cationic)	1801	7	928	4	194	4
(anionic)	508	22	474	28	784	43
Sephacryl S-300						
(DEAE anionic)	452	113	356	140	627	41

*Enzyme activities expressed as µmol product/h; **pNCS activity/4MUS activity.

Table 2. Comparison of properties of anionic and cationic pNCS- and 4MUS-sulfatase activities

	Rat		Mouse		Bovine	
	pNCS	4MUS	pNCS	4MUS	pNCS	4MUS
pH optimum						
Anionic	5.3	5.8	5.3	5.9	5.1	5.7
Cationic	5.7	5.7	5.9	5.9	5.5	5.7
pI*						
Anionic	4.7	4.7	4.0	4.0	4.8	4.8
Cationic	7.5	7.5	7.5	7.5	8.4	8.4
R_m**						
Anionic	0.44	0.44	0.40	0.40	0.39	0.39
Cationic	0.36	0.36	0.26	0.26	—	—
t ½ (60 °C; min)						
Anionic	7	67	142	7	29	26
Cationic	67	66	114	111	36	28
Kinetics***						
Anionic	A	L	A	L	A	L
Cationic	L	L	L	L	L	L

*Major isozyme; **Relative electrophoretic activity with respect to bromphenol blue; ***L, linear; A, anomalous.

Table 3. Comparison of properties of anionic and cationic pNCS- and 4MU-sulfatase activities (continued)

	Rat		Mouse		Bovine	
	pNCS	4MUS	pNCS	4MUS	pNCS	4MUS
Inhibitors (% residual activity):						
0.2mM $AgNO_3$						
Anionic	28 ± 3	101 ± 8	10 ± 2	79 ± 1	0	77 ± 8
Cationic	102 ± 2	90 ± 3	108 ± 3	94 ± 4	110 ± 5	81 ± 7
20mM NaCN						
Anionic	95 ± 7	193 ± 6	94 ± 1	100 ± 4	114 ± 5	280 ± 9
Cationic	114 ± 3	172 ± 8	107 ± 5	104 ± 6	112 ± 4	180 ± 6
2mM Na_2HPO_4						
Anionic	0	23 ± 1	7 ± 1	0	28 ± 1	16 ± 3
Cationic	0	15 ± 3	11 ± 2	4 ± 1	12 ± 4	6 ± 1
200mM NaCl						
Anionic	89 ± 3	51 ± 6	99 ± 1	78 ± 2	117 ± 5	139 ± 8
Cationic	67 ± 5	52 ± 8	69 ± 3	63 ± 8	76 ± 3	88 ± 6
25 mM Na_2SO_4						
Anionic	39 ±	15 ± 2	56 ± 2	7 ± 2	33 ± 1	26 ± 1
Cationic	0	12 ± 2	29 ± 4	5 ± 1	20 ± 4	9 ± 1

Table 4. Effects of rabbit anti-murine hepatic arylsulfatase B upon anionic and cationic pNCS- and 4MUS sulfatase activities

	Rat		Mouse		Bovine	
	pNCS	4MUS	pNCS	4MUS	pNCS	4MUS
% Résidual activity in supernatant:						
Anionic	93 ± 2	71 ± 3	94 ± 4	66 ± 3	102 ± 1	75 ± 2
Cationic	3	13	0	6 ± 3	77 ± 3	66 ± 2

*The following quantities of IgG were used for immunoprecipitation of anionic and cationic activities: rat = 12.5 µl; mouse = 5 µl; bovine = 50 µl.

tase activity displayed a more acidic pH optimum, was thermolabile, exhibited anomalous kinetics, was inhibited by silver, and was resistant to inhibition by chloride. These properties of rodent anionic pNCS-sulfatase activity are characteristic for arylsulfatase A. These data suggest that a significant portion of rodent anionic 4MUS-sulfatase activity may reside in an arylsulfatase B-like enzyme and not arylsulfatase A. This hypothesis is supported by precipitation of approximately one-third of anionic 4MUS-sulfatase activity by anti-murine arylsulfatase B IgG and failure of this polyclonal IgG preparation to precipitate pNCS-sulfatase activities from the same fractions (table 4).

Bovine anionic 4MUS-sulfatase activity possessed a pH optimum similar to that of arylsulfatase B, displayed linear kinetics, was resistant to inhibition by silver, and was partially precipitated by anti-arylsulfatase B IgG. However, bovine anionic 4MUS-sulfatase activity was not inhibited by NaCl, an inhibitor of arylsulfatase B. The half-denaturation times for bovine arylsulfatases A and B were comparable under the conditions utilized.

These data collectively suggest that: 1) anionic pNCS-sulfatase activity from both rodent and bovine liver is largely contributed by arylsulfatase A as previous investigators have reported, and 2) a significant proportion of rodent anionic 4MUS-sulfatase activity may be contributed by an arylsulfatase B-like isozyme. The situation for bovine anionic 4MUS-sulfatase activity is less clear, although some evidence suggests that an arylsulfatase B-like isozyme may be present in the bovine anionic 110 kD arylsulfatase fraction. The relative molecular weight of the anionic arylsulfatase B-like isozyme present in rodent preparations approximates that expected for a dimeric species of arylsulfatase B. Dimeric arylsulfatase B has been found in feline liver[15]. Failure to precipitate the majority of anionic 4MUS-sulfatase activity from rodent and bovine liver suggests that arylsulfatase A may be responsible for some 4MUS hydrolysis.

The suitability of 4MUS as a substrate for arylsulfatases A and B has been questioned by others[16]; however, many of the problems cited have been corrected by altering extraction methods and including polyvalent cations in the assay mixture to remove interfering anions[17,18]. In spite of these modifications, assay of arylsulfatase A in the presence of arylsulfatase B continues to depend upon the assumption that the two isozymes hydrolyze 4MUS with comparable efficiencies. The present report demonstrates that this assumption is invalid, and that 4MUS is a more suitable substrate for estimation of arylsulfatase B than arylsulfatase A. Since bovine and human tissues contain much more arylsulfatase A than arylsulfatase B activity, problems associated with utilization of 4MUS as substrate are minimized. However, rodent tissues contain a 4:1 excess of arylsulfatase B activity, and interference by arylsulfatase B and B-like isozymes with estimation of arylsulfatase A activity when using 4MUS as substrate is a major problem.

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