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SOLUBLE ARYLSULFATASES OF HUMAN BRAIN AND SOME CHARACTERISTICS OF THE BRAIN-SPECIFIC ARYLSULFATASE B_m

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Summary

The brain-specific arylsulfatase B_m (aryl-sulfate sulfohydrolase, EC 3.1.6.1) was demonstrable in human and monkey brain. Arylsulfatases A, B and B_m were separated employing DEAE-cellulose chromatography. There was a distinct difference in the proportion of the sulfatases in infant and adult human brain.

Arylsulfatase B_m after concanavalin A-Sepharose chromatography showed the property of binding to Sephadex G-200 totally. Several dissociating agents failed to elute the enzyme from the bound form. Under similar conditions arylsulfatase A did not show any binding to Sephadex. On treatment with *Escherichia coli* alkaline phosphatase adult human brain arylsulfatase B_m but not arylsulfatase A was converted into a less acidic, presumably dephosphorylated form that did not bind to DEAE-cellulose. Monkey brain arylsulfatase B_m showed a similar susceptibility to *E. coli* phosphatase treatment. Inorganic phosphate and serine phosphate but not mannose 6-phosphate could inhibit this dephosphorylation. There were differences in the susceptibilities to alkaline phosphatase treatment of the arylsulfatase B_m from infant and adult human brain. Endogenous phosphatase also seemed to have a role on the phosphorylated state of arylsulfatase B_m.

Introduction

The soluble arylsulfatases (aryl-sulfate sulfohydrolase, EC 3.1.6.1) have been isolated and characterized from various mammalian tissues (for review see Refs. 1—3). Arylsulfatases A and B, differentiated by their properties, have been purified from several human tissues and human urine and their properties

studied [4—7]. Their role in metachromatic leukodystrophy and Maroteaux-Lamy syndrome has been emphasized [8,9]. Because of their involvement in such human genetic disorders a study of the characteristics of arylsulfatases from human tissues becomes relevant.

Stevens et al. [10] reported that the human brain contains a minor anionic form of arylsulfatase, designated arylsulfatase B_m which was similar to arylsulfatase B in several characteristics but different from both arylsulfatase A and B in its elution pattern from DEAE-cellulose. It was also noticed by these workers that arylsulfatase B_m was present in significant amounts only in the brain but not in other tissues such as liver, kidney, testis and placenta.

The physiological significance of arylsulfatase B_m in brain is not clearly understood. It has been hypothesized that arylsulfatase B_m may be derived from arylsulfatase B as a result of post-ribosomal modification and that its predominance in brain suggests a special role for this enzyme in neural tissue [10]. We report here the characteristics of the arylsulfatases isolated by DEAE-cellulose chromatography from adult and infant human brain and also some characteristics of the brain-specific arylsulfatase B_m.

Materials and Methods

Nitrocatechol sulfate was prepared by the method of Dodgson and Spencer [11]. Sephadex and Sepharose were obtained from Pharmacia Fine Chemicals, Sweden. DEAE-cellulose (Whatman DE-32) was from Whatman, U.S.A. Calf intestinal alkaline phosphatase type I, *E. coli* alkaline phosphatase type III, 4-methyl umbelliferyl sulfate, mannose 6-phosphate, serine *O*-phosphate, dextran (200—275 000) and α-casein were from Sigma Chemicals, U.S.A. *Vibrio cholerae* neuraminidase from Behringwerke AG, F.R.G.; aquacide II from Calbiochem, U.S.A.; Triton X-100 from Rohm and Haas, U.S.A. Concanavalin A from *Canavalia gladiata* was prepared according to the method of Surolia et al. [12] and coupled to Sepharose 4B (activated at pH 8.5 in 0.1 M sodium bicarbonate buffer) according to the method of Cuatrecasas and Parikh [13]. The preparation contained 15 mg concanavalin A/ml Sepharose. All other chemicals were of the highest grade purity available.

Human brain cerebral cortex (adult and infant) from patients who had died of non-neurological disorders were collected from the mortuary and kept frozen at —18°C until use. Adult monkey (*Macaca radiata*) brain which was removed under nembutal anaesthesia was also stored under similar conditions.

Enzyme assays. For studying the distribution of the three arylsulfatase activities in the DEAE-cellulose fractions the following assay procedure was adopted. The incubation mixture contained 10 mM nitrocatechol sulfate, 0.2 M sodium acetate bufer, pH 5.0, and enzyme in a total volume of 0.2 ml. After incubation at 37°C for 10 min the reaction was stopped by adding 2.8 ml 1 N NaOH and the nitrocatechol released was read in a Klett Summerson colorimeter using filter 50. The same assay method was used to measure arylsulfatase activity in the crude homogenate and 12 000 × *g* supernatant (after dialysis against 0.02 M Tris-HCl, pH 7.4, before assay to prevent inhibition by endogenous phosphate).

Endogenous alkaline phosphatase activity was assayed using *p*-nitrophenyl

phosphate as substrate [14]. Proteolytic activity in calf intestinal and *E. coli* alkaline phosphatases was assayed according to the method of Kunitz [15] using casein as substrate.

Protein was estimated according to Lowry et al. [16] using crystalline bovine serum albumin as standard.

Preparation of the enzyme and DEAE-cellulose column chromatography. All operations were done at 0—4°C unless otherwise mentioned. 50 g of frozen tissue was homogenized with 9 vols. (450 ml) of 0.02 M Tris-HCl, pH 7.4 in a Waring blender for 6 min at maximum speed and centrifuged at 12 000 × *g* for 30 min. Solid $(NH_4)_2SO_4$ was added to the supernatant so obtained to 60% saturation. The mixture was centrifuged at 12 000 × *g* and the pellet was suspended in 12 ml 0.02 M Tris-HCl, pH 7.4, and dialyzed against the same buffer (3 l) for 12 h with two changes. After removing any sedimentable material at 12 000 × *g* for 30 min the dialyzed enzyme was loaded on a DEAE-cellulose column.

DEAE-cellulose column chromatography. The dialyzed $(NH_4)_2SO_4$ fraction was loaded on a column (26 × 2.1 cm) of DEAE-cellulose DE-32, preequilibrated with 0.02 M Tris-HCl, pH 7.4 at a flow rate of 20 ml/h. (The amount of protein used per ml of packed DEAE-cellulose was 3—3.8 mg.) The column was washed with 1 l of 0.02 M Tris-HCl, pH 7.4. Elution was carried out with a linear gradient, 0—0.6 M NaCl in 900 ml of the same buffer. Fractions of 10 ml were collected. Active fractions were pooled, dialyzed against the same buffer and concentrated against aquacide when needed.

Concanavalin A-Sepharose affinity chromatography. The pooled DEAE-cellulose fractions containing arylsulfatases A, B or B_m activity were loaded in separate experiments on a concanavalin A-Sepharose column (5.2 × 2.2 cm). Arylsulfatase A (33 mg protein) was loaded at pH 7.4 and arylsulfatases B and B_m (4 mg protein) at pH 6.0 in 0.02 M Tris-acetate buffer. The flow rate was 10 ml/h. The column was washed with 200 ml of 0.02 M Tris-HCl, pH 7.4/0.5 M NaCl and eluted with 0.5 M α-methyl glucoside/0.5 M NaCl in the same buffer at 25°C. The active fractions were pooled and dialyzed against the same buffer.

Binding to Sephadex. The aquacide concentrated eluate from either concanavalin A-Sepharose or from DEAE-cellulose column (0.2 ml) after dialysis against 0.02 M Tris-HCl, pH 7.4, was charged on a Sephadex G-200 column (5.7 × 0.7 cm) and washed with 6 ml of the same buffer at a flow rate of 2 ml/h. Fractions of 1 ml were collected.

Treatment of arylsulfatase B_m or A with alkaline phosphatase. Aquacide concentrated DEAE-cellulose fractions of arylsulfatase B_m or A were treated with either calf intestinal alkaline phosphatase (2 units/mg protein of arylsulfatase) or *E. coli* alkaline phosphatase (1 unit/mg protein of arylsulfatase). The reaction mixture containing 0.1 M Tris-acetate buffer, pH 8.0, alkaline phosphatase, 0.01 M $MgCl_2$ and arylsulfatase in a total volume of 2 ml was incubated at 37°C for 1 h and dialyzed immediately against 1 l of 0.02 M Tris-HCl, pH 7.4 for 4 h. It was then charged on a DEAE-cellulose column (9.8 × 0.8 cm), washed with 15 ml of 0.02 M Tris-HCl, pH 7.4 and eluted with 0.2 M NaCl for arylsulfatase B_m or 0.4 M NaCl for arylsulfatase A in the same buffer. (In initial experiments gradient elution with NaCl was used and it was found

that the unaltered arylsulfatase B_m or A was eluted by NaCl as a single peak at their respective concentration of NaCl. To simplify the procedure in the subsequent experiments a single step elution with NaCl was used as given here.) The flow rate was maintained at 5 ml/h and 2 ml fractions were collected. The substrate concentration used for assaying the fractions was 7.5 mM. There was about 15—20% loss of arylsulfatase B_m activity after its incubation with or without alkaline phosphatase.

Both *E. coli* and calf intestinal phosphatases had no detectable proteolytic activity under the above reaction conditions. *E. coli* phosphatase had no nitrocatechol sulfatase activity while that of calf intestinal phosphatase had less than 1% activity of the arylsulfatase used as substrate.

Polyacrylamide gel electrophoresis. Electrophoresis [17] was done using 0.1 ml of the aquacide concentrated and dialyzed enzyme fractions on 7% gels at 3 mA in 0.05 M Tris-glycine, pH 8.0, for 2 h when the tracking dye emerged from the gel. The gel was sliced into 4.5 mm thickness and each gel-slice was assayed by incubating with 0.1 ml of a solution containing 10 mM 4-methyl umbelliferyl sulfate in 0.5 M acetate buffer, pH 5.0, for 2 h at 37°C. The reaction was stopped by adding 2.8 ml of 0.4 M glycine/NaOH buffer, pH 10.5 and the fluorescence of 4-methyl umbelliferone was measured in a Hitachi 204-A fluorescence spectrometer [18].

Neuraminidase treatment. Pooled DEAE-cellulose fractions containing arylsulfatase B_m activity after aquacide concentration were incubated with *V. cholerae* neuraminidase (25 units/mg arylsulfatase protein) in 0.1 M acetate buffer, pH 5.5/0.01 M $CaCl_2$ for 1 h at 37°C, dialyzed against buffer and loaded on a DEAE-cellulose column as described for phosphatase treatment.

Results

Separation of the arylsulfatases

Typical profiles of arylsulfatases A, B and B_m from infant and adult human brain and monkey brain on the DEAE-cellulose column are depicted in Fig. 1. The identity of the arylsulfatases A, B and B_m was also confirmed by other experiments. Time vs. activity curves of the arylsulfatases A, B and B_m are shown in Fig. 2. Arylsulfatase A at varying protein concentrations showed the typical anomalous curves characteristic of arylsulfatase A [19]. With arylsulfatase B the reaction rate decreased beyond 30 min of incubation while arylsulfatase B_m activity was linear upto 4 h of incubation (Fig. 2). All the enzyme activities were linear for a period of 10 min of incubation. The differential inhibitions by silver ion of arylsulfatases have been shown [10]. At 0.2 mM $AgNO_3$ arylsulfatase A was inhibited about 81% while B was inhibited only about 7%. Arylsulfatase B_m was inhibited 34% under these conditions. At higher concentrations $AgNO_3$ inhibited all the arylsulfatases to a higher degree. Apparently arylsulfatases B and B_m seem to be more sensitive to inhibition by $AgNO_3$ when nitrocatechol sulfate instead of methyl umbelliferyl sulfate is used as substrate [10].

Table I shows the proportions of arylsulfatases A, B and B_m in adult and infant human brain and adult monkey brain. It was observed that the proportion of arylsulfatase A was always higher and that of arylsulfatase B less in the

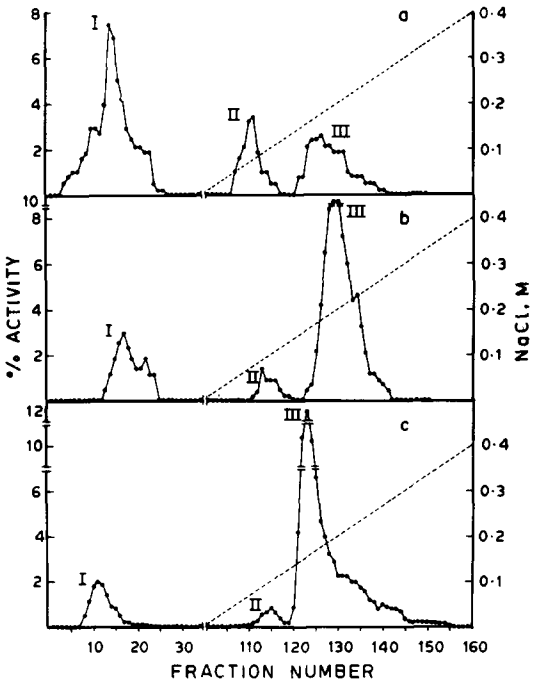

Fig. 1. DEAE-cellulose ion-exchange chromatography of the $(NH_4)_2SO_4$ fractions of infant and adult human brain and monkey brain. The total activity from all fractions eluted from the column is taken as 100%. Peaks I, II, III correspond to arylsulfatases B, B_m and A, respectively. a. Infant human brain (from child immediately after birth). b. Adult human brain (from a 45-year-old male). c. Adult monkey brain.

adult brain as compared to the infant brain. Also, the proportion of arylsulfatase B_m was higher in infant brain as compared to adult brain. The proportions of the arylsulfatase in adult monkey brain was comparable to adult human brain. Although these experiments were done using a 12 000 × *g* supernatant fraction, we also investigated DEAE-cellulose elution patterns from a

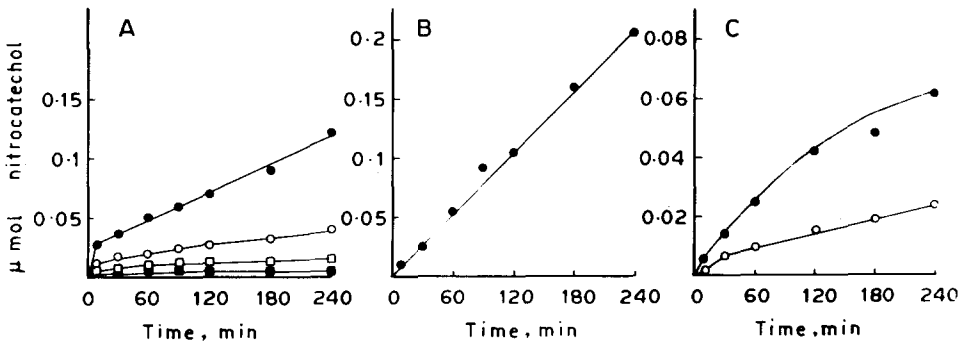

Fig. 2. Time vs. activity curves of DEAE-cellulose fractions of human brain arylsulfatases. A. Arylsulfatase A: ●———●, 248 μg protein; ○———○, 124 μg protein; □———□, 62 μg protein; ■———■, 31 μg protein. B. Arylsulfatase B_m: ●———●, 56 μg protein. C. Arylsulfatase B: ●———●, 26 μg protein ○———○, 13 μg protein.

TABLE I

PERCENTAGE ACTIVITIES OF ARYLSULFATASES A, B_m AND B AFTER DEAE-CELLULOSE CHROMATOGRAPHY

The description of the infant and adult human brain are as given under Fig. 1. Results are given from different batches of enzyme prepared. The total activity from all fractions eluted from the DEAE-cellulose column is taken as 100%.

Source	Batch number	Arylsulfatase activity (%)		
		B	B_m	A
Infant human brain	Batch I	31.5	11.8	56.6
	Batch II	57.1	16.4	26.5
	Batch III	42.6	13.6	43.8
	Batch IV *	49.8	13.2	39.0
Adult human brain	Batch I	20.3	5.8	73.8
	Batch II	11.8	5.3	82.9
	Batch III	34.2	3.7	62.1
Adult monkey brain	Batch I	12.0	3.7	84.3

* Batch IV enzyme of infant human brain was from a 105 000 × *g* supernatant.

105 000 × *g* supernatant fraction of infant human brain (Table I). Essentially similar results were obtained.

pH optima and inhibition studies

The pH optima of arylsulfatases A, B and B_m were respectively 5.0, 5.5 and 5.5. The K_m values of the three enzymes were 1.0, 3.33 and 2.86 mM, respectively. Both SO_3^{2-} and PO_4^{3-} at 0.5 mM or above were inhibitory to all the three enzymes. At 0.5 mM of sodium sulfite arylsulfatases A, B and B_m were inhibited 46, 70 and 48%, respectively. Potassium phosphate at the same concentration inhibited the enzymes 91, 92 and 89%, respectively.

Behaviour of the arylsulfatases on concanavalin A-Sepharose

Passage of arylsulfatases B and B_m through concanavalin A-Sepharose column as given under Materials and Methods indicated that binding was complete at pH 6.0, but not at pH 7.4. Arylsulfatase A was fully bound by the column at pH 7.4. Elution of the enzyme by α-methyl glucoside showed that the percentage recovery of arylsulfatase B was only 45% and that of B_m and A almost 100%. Attempts to elute the remaining arylsulfatase B from the column with ethylene glycol (50%, v/v), α-methyl mannoside (0.5 M) or a higher concentration (1.0 M) of α-methyl glucoside were unsuccessful. On testing the gel for B activity it was found that the remaining activity was still retained on the column.

Sephadex G-200 binding

An attempt was made to purify the aquacide concentrated arylsulfatase B_m after concanavalin A-Sepharose chromatography by passing through a Sephadex G-200 column. It was found that no enzyme activity could be recovered from the gel. This phenomenon was subjected to further studies. When the enzyme was passed through a small Sephadex G-200 column (see

Materials and Methods) only about 2% activity was detectable in the effluent and the remaining activity was found bound to the gel (verified by incubating the gel with nitrocatechol sulfate in the assay mixture). The bound enzyme was not eluted by several agents such as 1.0 M NaCl (to reduce electrostatic interaction), 0.5 M glucose, 0.5 M glucose plus 0.5 M NaCl, 0.5 M α-methyl glucoside (to nullify any biospecific interaction between the enzyme and Sephadex), Dextran (10 mg/ml), 0.5 M KSCN (to reduce the hydrophobic interaction), 1.0 M urea, a mixture of tyrosine, phenylalanine and tryptophan, each 0.1 M in 0.02 M Tris-HCl, pH 7.4 (to minimise aromatic adsorption) 1% (v/v) Triton X-100, EDTA (10^{-3} M), concanavalin A solution (1 mg/ml), 0.02 M Tris-HCl, pH 7.4 at 4°C and 25°C, 0.02 M sodium acetate buffer, pH 5.0, and mechanical homogenization in a Potter-Elvehjem homogenizer. (None of these reagents used for elution was significantly inhibitory to arylsulfatase B_m except KSCN and concanavalin A solution which inhibited 50 and 20%, respectively.) Even after these treatments the enzyme activity was detectable in the gel.

Under similar conditions of Sephadex G-200 gel treatment of arylsulfatases A and B purified by concanavalin A-Sepharose column chromatography, it was observed that more than 80 and 40%, respectively of the two enzymes were recoverable in the effluent. Although recovery appeared to be somewhat low, particularly for arylsulfatase B, in neither of these cases was there any detectable sulfatase activity associated with the gel. The possibility is considered that some loss in activity occurred, particularly for arylsulfatase B, during the gel filtration.

Attempts were made to explore the possible reasons for the binding of arylsulfatase B_m to Sephadex G-200. It has been suggested that aggregation of arylsulfatase B at low ionic strength results in its binding to Sephadex [20]. Centrifugation of the concanavalin A-Sepharose eluates of arylsulfatases B and B_m at $12\,000 \times g$ resulted in the sedimentation of both enzyme activities to the extent of 30 and 100%, respectively, while arylsulfatase A was not sedimentable. This suggested a probable aggregation of the arylsulfatase B_m after concanavalin A-Sepharose treatment. Furthermore, arylsulfatases B and B_m before passage through concanavalin A-Sepharose were not sedimentable at $12\,000 \times g$ and almost 85% of the activity was recovered in the supernatant. When passed through the Sephadex G-200 column, they were recoverable to the extent of about 40% in the effluent and a major portion of the remaining B activity, but not B_m activity, was eluted by 1 M NaCl.

The thermal stability of arylsulfatase B_m in the free form and bound to Sephadex G-200 was examined by preincubating for varying periods at 55°C. There was no significant difference in the thermal stabilities and both forms lost considerable activity above 60°C.

Phosphatase treatment

Alkaline phosphatase treatment of the adult human brain and monkey brain arylsulfatase B_m resulted in significant changes in their elution pattern from DEAE-cellulose.

Both *E. coli* as well as calf intestinal alkaline phosphatase treatment resulted in the formation of a new form of arylsulfatase B_m which did not bind to

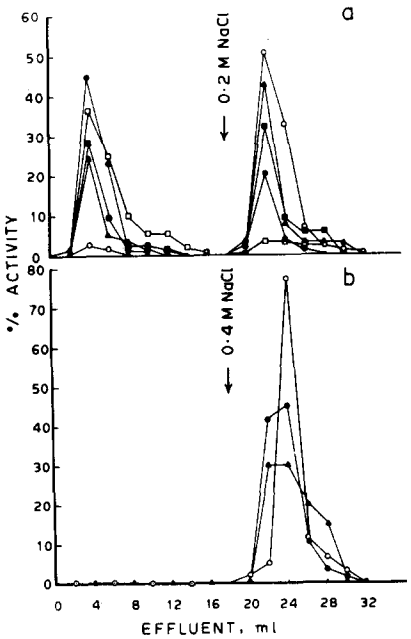

Fig. 3. Profile of arylsulfatase B_m (a) and arylsulfatase A (b) on DEAE-cellulose column before and after *E. coli* alkaline phosphatase treatment. For experimental details see Materials and Methods. a. Arylsulfatase B_m without incubation ○———○; incubated without *E. coli* alkaline phosphate ▲———▲; incubated with *E. coli* alkaline phosphatase, ●———●; incubated with *E. coli* phosphatase in presence of 10 mM mannose 6-phosphate, □———□; incubated with *E. coli* phosphatase in presence of 10 mM serine *O*-phosphate ■———■. b. Arylsulfatase A incubated without alkaline phosphatase, ○———○; incubated with calf intestinal alkaline phosphatase, ●———●; incubated with *E. coli* alkaline phosphatase, ▲———▲.

DEAE-cellulose. Preliminary experiments showed both the phosphatases to be equally effective under the experimental conditions used. Fig. 3a shows the DEAE-cellulose chromatographic profile of adult human brain arylsulfatase B_m subjected to *E. coli* alkaline phosphatase treatment. Almost 96% of the B_m enzyme without any incubation was bound to DEAE-cellulose but when incubated for 1 h at 37°C about 36% of the enzyme was found unbound to the DEAE-cellulose (Fig. 3a; see also Table II). When incubated with *E. coli* alkaline phosphatase about 71% of the enzyme was unbound to the column (Fig. 3a). It is presumable that dephosphorylation of the enzyme resulted in converting it into a form that did not bind to DEAE-cellulose. Since mannose 6-phosphate is suggested to be a phosphorylated residue in many glycoprotein enzymes [21,22] and serine *O*-phosphate in phosphoproteins [23] we examined the effect of these compounds at 10 mM on the dephosphorylation of arylsulfatase B_m. While mannose 6-phosphate did not inhibit the dephosphorylation of the enzyme serine phosphate showed approx. 60% inhibition of the dephosphorylation of the enzyme (Fig. 3a). Both serine phosphate and mannose phosphate liberated inorganic phosphate to a concentration of 4.75 mM and 1.75 mM, respectively under these conditions. However, at 5 mM of inorganic phosphate there was only about 45% inhibition of the dephosphorylation of arylsulfatase B_m. Therefore the inhibition by serine phosphate

TABLE II

ALKALINE PHOSPHATASE TREATMENT OF ARYLSULFATASE B_m

Experimental details of alkaline phosphatase treatment and DEAE-cellulose chromatography of arylsulfatase B_m are given under Materials and Methods. Alkaline phosphatase was assayed as given under Materials and Methods.

Source of arylsulfatase B_m	Expt. No.	Endogenous alkaline phosphatase in B_m preparation nmol *p*-nitrophenol per minute per ml	Treatment of arylsulfatase B_m	Percentage activity in the dephosphorylated fraction	Percentage activity in the phosphorylated fraction
Adult human brain	1	12.5	No incubation	4	96
			Incubated for 1 h	36	64
			Incubated for 1 h with *E. coli* alkaline phosphatase	71	29
Monkey brain	1	1.55	No incubation	0	100
			Incubated for 1 h	2	98
			Incubated for 1 h with *E. coli* alkaline phosphatase	30	70
	2	1.98	Incubated for 1 h	2	98
			Incubated for 1 h with *E. coli* alkaline phosphatase	25	75
			Incubated for 1 h with *E. coli* phosphatase + 0.1 M potassium phosphate pH 8.0	4	96
Infant human brain	1	19.62	Incubated for 1 h	27	73
			No incubation but enzyme stored at 4°C for 45 days	22	77
			Incubated for 1 h with calf intestinal alkaline phosphatase	29	71

must be at least partly due to the compound itself rather than the phosphate liberated from it. Fig. 3b shows the effect of alkaline phosphatase on adult brain arylsulfatase A. There was no evidence of change in the elution pattern of the enzyme.

Since incubation of the arylsulfatase B_m preparation without exogenous alkaline phosphatase also showed changes in the binding characteristics of arylsulfatase B_m to DEAE-cellulose, we measured the endogenous alkaline phosphatase in some of the B_m preparations. Table II shows the effect of incubation of the B_m enzyme from adult human, monkey and infant human brain with or without alkaline phosphatase. Also shown in the table is the endogenous alkaline phosphatase activity. It is seen that when there is an increased endogenous alkaline phosphatase activity a greater proportion of dephosphorylation occurs during incubation or during long storage of the enzyme at 4° C without any added alkaline phosphatase. Monkey brain B_m preparation which had very low endogenous alkaline phosphatase activity did not undergo any significant dephosphorylation on incubation. It is also noteworthy that dephosphorylation by *E. coli* phosphatase could be completely inhibited by 0.1 M inorganic phosphate (Table II). It is also seen from the table that in addition to the dephosphorylation by the endogenous alkaline phosphatase, the *E. coli* phosphatase alone was able to dephosphorylate about 23—35% of the enzyme from both adult human and monkey brain. However, in the case of infant human brain enzyme, exogenous phosphatase had no detectable effect in dephosphorylation.

Gel electrophoresis of the phosphorylated and dephosphorylated arylsulfatase B_m

The adult human brain arylsulfatase B_m before *E. coli* phosphatase treatment and the two fractions (phosphorylated and dephosphorylated) after *E. coli* phosphatase treatment followed by DEAE-cellulose chromatography were subjected to gel electrophoresis. After electrophoresis when the gels were incubated with 4-methyl umbelliferyl sulfate and viewed under long wavelength ultraviolet light [18] diffuse fluorescent bands of the enzyme activity were visible. The dephosphorylated B_m was found to have a lower migration as compared to the phosphorylated enzyme. The gels after electrophoresis, were cut into slices and incubated with 4-methyl umbelliferyl sulfate as given under Materials and Methods. Arylsulfatase B_m before *E. coli* phosphatase treatment as well as the phosphorylated fraction obtained from the DEAE-cellulose showed a peak of activity in the second slice from the origin at the cathode end. On the other hand, the dephosphorylated form of B_m from the DEAE-cellulose column showed the peak of activity in the first gel slice. There was some diffusion of all the B_m enzyme bands on the gel as observed by Stevens [18].

Kinetic properties of phosphorylated and dephosphorylated arylsulfatase B_m

There was no significant difference between the phosphorylated and dephosphorylated B_m enzymes in their pH optima and K_m value. The substrate concentration-activity curves showed a marked decrease in activity of both dephosphorylated and phosphorylated B_m above a substrate concentration of 7.5 mM unlike arylsulfatase B (Fig. 4).

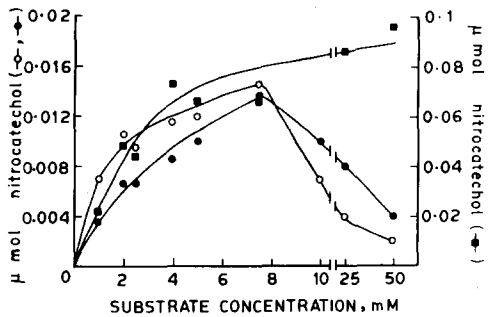

Fig. 4. Substrate concentration vs. activity curves of arylsulfatase B (■———■), phosphorylated arylsulfatase B_m (●———●), and dephosphorylated arylsulfatase B_m (○———○). The latter two enzymes were obtained by DEAE-cellulose chromatography of *E. coli* phosphatase-treated arylsulfatase B_m.

Neuraminidase treatment

On incubating arylsulfatase B_m with neuraminidase at pH 5.5 it lost about 85% activity. Hence no further experiment on the effect of neuraminidase was done. It was also observed that the B_m enzyme was less stable at and below pH 5.5 when incubated at 37°C.

Discussion

In the present studies arylsulfatases A, B and B_m from human brain have been separated by DEAE-cellulose chromatography. There are definite differences between the adult and infant brain in the proportions of the three arylsulfatases. The presence of the brain specific arylsulfatase B_m is shown in the monkey brain also.

The properties of arylsulfatase B_m such as time vs. activity curves and pH optimum are more similar to those of arylsulfatase B. The B_m enzyme after concanavalin A-Sepharose chromatography is shown to bind to Sephadex G-200. In this respect also it has some similarity to arylsulfatase B, which is reported to have a tendency to bind to Sephadex [20].

The irreversible binding of arylsulfatase B_m to Sephadex G-200 after concanavalin A-Sepharose purification needs some consideration. It has been indicated that small amounts of concanavalin A are eluted when an enzyme is passed through and eluted from concanavalin A-Sepharose column [24]. If such were the case, one would expect the enzyme to bind to Sephadex through the concanavalin A. However, this possibility appears remote in the case of arylsulfatase B_m because glucose or α-methyl glucoside failed to elute the enzyme from the Sephadex G-200. Furthermore arylsulfatase A obtained from concanavalin A-Sepharose column did not bind to Sephadex G-200.

Arylsulfatase B_m after concanavalin A-Sepharose purification shows a tendency to sediment at 12 000 × *g* and the possibility of sedimentation of the enzyme on Sephadex G-200 was considered. Since mechanical homogenization of the gel failed to release the enzyme from the gel, the sedimentation of the enzyme on the gel may be ruled out. The property of arylsulfatase B_m to sediment at 12 000 × *g* after concanavalin A-Sepharose treatment suggests that an aggregation of the enzyme occurs after this step and it is possible that this

aggregable nature of the enzyme also contributes towards its binding to Sephadex G-200 as suggested by Allen and Roy [20]. This phenomenon has also some similarity to the reported aggregation of rRNA and its immobilisation on agarose [25]. What forces are responsible for the binding of arylsulfatase B_m to Sephadex is not clear because several dissociating agents failed to elute the enzyme from the bound form.

The ability of alkaline phosphatase to convert arylsulfatase B_m to a less acidic form unable to bind to DEAE-cellulose suggests that phosphate groups impart the negative charge on the enzyme. That the conversion is due to a phosphatase is also supported by the observation that inorganic phosphate completely inhibits the effect of *E. coli* phosphatase on the B_m enzyme (Table II). It is also noteworthy that where endogenous alkaline phosphatase activity is high in B_m preparations, incubation at 37° C or long storage at 4° C results in the partial dephosphorylation of the enzyme. It is conceivable that the alkaline phosphatases are able to dephosphorylate arylsulfatase B_m. Whether the endogenous alkaline phosphatase as well as the exogenous alkaline phosphatase dephosphorylate the same or different types of phosphate residues in B_m is not clear. The ability of serine phosphate to inhibit the dephosphorylation is an indication that B_m may be a phosphoprotein containing serine phosphate residues. The endogenous alkaline phosphatase activity measured using *p*-nitrophenyl phosphate, may not be fully representative of phospho-protein phosphatase activity, but there is evidence to indicate that *p*-nitrophenyl phosphatase activity and casein phosphatase activity approximately parallel each other in different tissues of the rat [26]. Moreover, *E. coli* phosphatase has also been effectively used to dephosphorylate casein [27] and other phosphoproteins [28]. In any case, the nature of the phosphate residues in the enzyme should await further studies with a purified preparation of arylsulfatase B_m. It is unlikely that by dephosphorylation arylsulfatase B_m is converted to arylsulfatase B because the substrate concentration-activity curves of dephosphorylated arylsulfatase B_m and arylsulfatase B differ significantly (Fig. 4).

The physiological significance of arylsulfatase B_m particularly its predominance in brain [10] is not clear. It is shown to share many properties in common with arylsulfatase B including its action on the substrate, UDP-*N*-acetyl galactosamine 4-sulfate [10].

The evidence that B_m may be a phosphorylated protein, presented here, would suggest that phosphorylation-dephosphorylation mechanisms may greatly influence the predominance and function of arylsulfatase B_m in mammalian tissue. The ineffectiveness of exogenous phosphatase to dephosphorylate infant human brain arylsulfatase B_m would suggest age-dependent changes in the phosphorylated residues present in the B_m enzyme.

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