

PARTIAL PURIFICATION, PROPERTIES AND GLYCOPROTEIN NATURE OF ARYLSULPHATASE B FROM SHEEP BRAIN

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Abstract A simple method has been developed for the partial purification of arylsulphatase B from sheep brain. This includes concanavalin A-Sepharose affinity chromatography and ionic strength-dependent binding and dissociation of the enzyme with Dextran Blue; by these methods the enzyme was purified 1344-fold with 10% recovery. The partially purified enzyme was shown to be a glycoprotein and its kinetic properties were compared with that of purified arylsulphatase A from the same source.

ARYLSULPHATASE B is known to be a lysosomal enzyme and it has been investigated over the last 30 years. It has been partially purified from liver as well as brain and some of its properties have been studied (ALLEN & ROY, 1968; BLESZYNSKI *et al.*, 1969; HARRINATH & ROBINS, 1971; DODGSON & WYNN, 1958). Arylsulphatase B is known to exist in multiple forms and the recent work of BLESZYNSKI & ROY (1973) have shown that ox brain enzyme has as many as seven isoenzymes. Even though arylsulphatase B has been detected in almost all tissues, a physiological role for this enzyme has not been elucidated. Recently STUMPF *et al.* (1973) observed that in one type of mucopolysaccharidosis (Maroteux-Lamy Syndrome) where there is an accumulation of dermatan sulphate in certain organs and excretion in urine, there was a complete absence of arylsulphatase B activity. This has been supported by the work of O'BRIEN *et al.* (1974) who also observed a deficiency of sulphatase activity in cultured fibroblasts derived from these patients. These observations suggest that arylsulphatase B may have a role in the cleavage of sulphate from sulphated glycosaminoglycans.

Recent work from our laboratory has shown that most of the brain lysosomal acid hydrolases are glycoproteins and that they bind with concanavalin A (BISHAYEE & BACHHAWAT, 1974). This is a lectin which can bind with glycoproteins having alpha-mannosyl or alpha-glucosyl residues and its binding property has been utilized for the purification of certain glycoprotein enzymes. Arylsulphatase A is known to be a lysosomal enzyme and the purified preparation from ox liver (GRAHAM & ROY, 1973) and sheep brain (BALASUBRAMANIAN & BACHHAWAT, 1975) has been shown to be a glycoprotein. Arylsulphatase B is also a lysosomal enzyme but so far there is no report on the glycoprotein nature of this enzyme. Earlier, GOLD-

STONE *et al.* (1971) showed that most of the kidney lysosomal enzymes are glycoproteins and claimed that neuraminidase treatment converted arylsulphatase A to a B form. Later GRAHAM & ROY (1973) showed that neuraminidase treatment does not convert arylsulphatase A to B and there was no change in the physical or chemical properties of the enzyme. Recently GOLDSTONE & KOENIG (1974) showed that neuraminidase treatment of the lysosomal enzymes results in a change from an acidic to a basic form of the enzyme. Taking into consideration all the above observations it was necessary to purify both arylsulphatases A and B from the same source, compare their properties and demonstrate their glycoprotein nature. It has already been shown that purified arylsulphatase A was a glycoprotein and there was no change in the properties of the enzyme by neuraminidase treatment (BALASUBRAMANIAN & BACHHAWAT, 1975).

The present study describes a simple method for the partial purification of arylsulphatase B from sheep brain. This includes affinity chromatography on concanavalin A-Sepharose and the ionic strength-dependent binding and dissociation of the enzyme with Dextran Blue. By these methods a highly purified arylsulphatase B was obtained which has been shown to be a glycoprotein and its properties were compared to that of arylsulphatase A from the same source.

MATERIALS AND METHODS

Sephadex G-200, Dextran Blue and Sepharose 6B were purchased from Pharmacia, Uppsala, Sweden. γ -Globulin, crystalline bovine serum albumin, ovomucoid trypsin inhibitor, cytochrome *c* and Coomassie Brilliant Blue were obtained from Sigma Chemical Co., U.S.A. Acrylamide and *N-N*-bis acrylamide were obtained from Eastman Organic Chemical, U.S.A. All other chemicals used were of analytical grade.

Methods

The nitrocatechol sulphate was prepared according to the method of DODGSON & SPENCER (1957). It was

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observed that even after recrystallization of nitrocatechol sulphate the preparation contained some impurity. The contaminating polymer was removed by gel filtration according to the method of AHMAD & BACHHAWAT (unpublished). In this method an aqueous solution of nitrocatechol sulphate was passed through a Sephadex G-10 column and the material in the inner volume was collected and freeze-dried. The polymer containing material which was eluted in the void volume was not acted upon by arylsulphatase A. The polymer was not further characterized. Concanavalin A was prepared from jack bean according to the method of SURIOLIA *et al.* (1973) and the concanavalin A-Sepharose was prepared as described by BISHAYEE & BACHHAWAT (1974) except that Sepharose 6B was used instead of Sepharose 4B. This preparation had 16 mg of concanavalin A bound per ml of packed Sepharose 6B as judged by the protein content of the washings. Purified arylsulphatase A was prepared as described by BALASUBRAMANIAN & BACHHAWAT (1975).

Protein estimation. Protein was determined by the method of LOWRY *et al.* (1951) with crystalline bovine serum albumin as standard.

Carbohydrate analysis of enzyme. The neutral sugar content of the enzyme was estimated by the phenol sulphuric acid method of ROUGHAN & BATT (1968) using galactose as standard. For identification of neutral sugars the enzyme (360 μ g protein) was hydrolysed at 100°C with 1 M-HCl for 5 h in a sealed tube and processed according to the method of SPIRO (1966). The sugars were identified by paper chromatography using the solvent system of MUKHERJEE & SRI RAM (1964). After drying the paper, the reducing sugar spots were identified by staining with alkaline silver nitrate. The sialic acid content of the enzyme was determined according to the thiobarbituric assay of WARREN (1959) as modified by SAIFER & GERSTENFELD (1962). For sugar estimation and sugar analysis the possible sugar leached from the Sephadex G-200 column was separated from the enzyme as follows. The enzyme solution was dialysed against saturated ammonium sulphate at 0°C whereby the enzyme protein was precipitated. It was centrifuged and the precipitate was dissolved in 0.001 M-acetate buffer pH 6 and dialysed against the same buffer.

Gel electrophoresis. Polyacrylamide gel electrophoresis with 7.5% gels was carried out by the method of ORNSTEIN & DAVIS (1962) using a Canalco electrophoretic apparatus in 0.075 M- β -alanine-acetic acid buffer, pH 4.5, for 4 h using 3 mA/tube. The gels were stained with Coomassie Brilliant Blue according to the method of CHRAMBACH *et al.* (1967). Periodic acid-Schiff-staining for glycoproteins was done according to KAPITANY & ZEBROWSKI (1973).

Molecular weight determination. The molecular weight of arylsulphatase B was determined by gel filtration by the method of ANDREWS (1965) using Sephadex G-200. The column was equilibrated with 0.02 M acetate buffer pH 6 containing 1 M-NaCl. A flow rate of 15 ml/h was maintained and 2 ml fractions were collected. The column operation was carried out at 23°C. The column was calibrated with cytochrome *c*, γ -globulin, ovomucoid trypsin inhibitor, bovine serum albumin and ovalbumin.

Arylsulphatase assay. Total arylsulphatase activity was assayed according to the method of WOORWOOD *et al.* (1973). During the first two steps of the enzyme purification an approximation of the total combined activities of arylsulphatase A and B present was obtained as follows. The incubation mixture consisted of 0.2 ml of 0.02 M-nitrocatechol sulphate in 0.5 M-sodium acetate solution adjusted

with acetic acid to pH 5.6. Incubation was done at 37°C for 10 min after which the reaction was stopped by the addition of 2.5 ml of 1 M-NaOH and the colour developed was measured in the Klett colorimeter using a no. 50 filter. Since the zinc acetate fractionation partially separates arylsulphatases A and B as observed by FAROOQUI & BACHHAWAT (1971), in the steps following zinc acetate precipitation, the enzyme was assayed at pH 6 instead of pH 5.6. One unit of enzyme activity is defined as the amount of enzyme required to produce one μ mol of nitrocatechol from *p*-nitrocatechol sulphate in 10 min under the assay conditions. The specific activity was expressed as units/mg protein.

Purification of arylsulphatase B

Preparation of crude homogenate and alcohol extraction. Sheep brain obtained immediately after killing was chilled on ice, freed from adhering membrane and blood and was stored at -18°C until used. Frozen tissue (1100 g) was homogenized with 2200 ml of 0.03 M-Tris-acetate buffer, pH 7.4, in a Waring blender for 2 min. This crude homogenate (2950 ml) contained 82.6 g protein. This was then subjected to alcohol extraction with constant mechanical stirring. Ethanol was added dropwise to a final concentration of 20% v/v and after 20 min the mixture was centrifuged at 12,000 *g* for 60 min. A small portion of the supernatant fluid was dialysed against 0.001 M-Tris-acetate buffer, pH 7.4, for the assay of the enzyme. The alcohol supernatant fluid (1760 ml) contained 12.1 g protein.

Zinc acetate fractionation and pH 5.0 precipitation. This was then subjected to zinc acetate fractionation. Zinc acetate solution (0.2 M) was added dropwise to a final concentration of 0.005 M with constant mechanical stirring. The above steps were carried out at 0°C. After 20 min the mixture was centrifuged at 12,000 *g* for 50 min. This step gave a partial separation of arylsulphatases A and B. The reddish precipitate which contained most of the B enzyme was suspended and homogenized in 0.1 M-citrate buffer, pH 7. This suspension was dialysed exhaustively against 0.001 M-Tris-acetate buffer, pH 7.4. Zinc acetate precipitate (204 ml) contained 4.48 g protein and was then subjected to pH 5.0 precipitation. An equal volume of 1 M-sodium acetate-acetic acid buffer, pH 5, was added to the zinc acetate precipitate and kept at 37°C for 15 min and centrifuged at 12,000 *g* for 30 min. This supernatant fluid was then dialysed exhaustively against 0.001 M-acetate buffer, pH 6, whereby some precipitate was formed. This was centrifuged at 12,000 *g* for 30 min and the supernatant fluid collected. This was kept overnight at room temperature which resulted in further precipitation of some of the inert proteins. This was again centrifuged at 12,000 *g* for 30 min and the pH 5.0 supernatant fluid was collected.

Concanavalin A-Sepharose affinity chromatography. To supernatant fluid obtained (465 ml) solid sodium chloride was added to a final concentration of 0.2 M and passed through a concanavalin A-Sepharose column previously equilibrated with 0.02 M-acetate buffer, pH 6, containing 0.2 M-sodium chloride. The column was run at room temperature (28°C) and the flow rate was maintained at 60 ml/h. The size of the concanavalin A-Sepharose column depends on the protein content of the supernatant fluid. On the assumption that 20% of the total protein to be glycoprotein, a 10 ml column was used. After loading the pH 5 supernatant, the column was washed with 5 bed volumes of 0.02 M-acetate buffer, pH 6, and then successively washed with 10% methylglucoside in 0.02 M-acetate

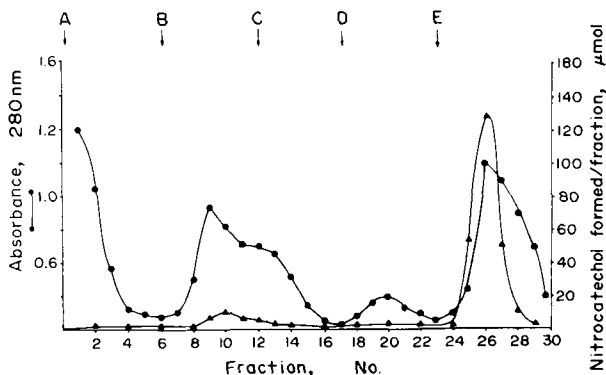


FIG. 1. Elution profile of arylsulphatase B from concanavalin A-Sepharose column. Fractions (5 ml) were collected and assayed for enzyme activity as described in Methods. (A) Washing the column with 0.02 M-acetate buffer pH 6, (B) washing with 10% methyl glucoside in 0.02 M-acetate buffer pH 6, (C) washing with 0.02 M-acetate buffer pH 6, (D) washing with 1 M-sodium chloride in 0.02 M-acetate buffer, pH 6, (E) elution with 10% methyl glucoside and 1 M-sodium chloride in 0.02 M-acetate buffer pH 6. (●) Absorbancy at 280 nm. (▲) Arylsulphatase activity.

buffer, pH 6. 0.02 M acetate buffer, pH 6. 1 M-NaCl in 0.02 M-acetate buffer, pH 6. The enzyme was eluted from the column with 0.02 M-acetate buffer, pH 6 containing 10% methylglucoside and 1 M-sodium chloride. The elution pattern of the concanavalin A-Sepharose column is shown in Fig. 1. The active fractions were pooled and dialysed against 0.001 M-acetate buffer, pH 6. This had 4.65 mg protein in 25 ml. This was then concentrated by dialysis against saturated ammonium sulphate whereby all the proteins were precipitated. This was centrifuged at 12,000 *g* for 30 min and the precipitate was dissolved in the minimum amount of 0.005 M-acetate buffer, pH 6, and dialysed against the same buffer.

Dextran Blue binding and dissociation: Dextran Blue (5%) was added with stirring to a final concentration of 0.5% to the dialysed ammonium sulphate concentrated enzyme and kept overnight at 6°C. This was then placed on a

column of Sephadex G-200 (45 × 0.8 cm) previously equilibrated with 0.005 M-acetate buffer, pH 6. Fractions (2 ml) were collected and the flow rate was maintained at 15 ml per h. The enzyme was shown to Dextran Blue and appeared together with it as bound in Fig. 2. The fractions from tubes 7 to 10 were pooled and to this solid sodium chloride was added a final concentration of 1 M. This solution (8 ml) was then placed on a column of Sephadex G-200 (65 × 1.5 cm) previously equilibrated with 0.02 M-acetate buffer, pH 6, containing 1 M-sodium chloride. The flow rate was maintained at 15 ml per h and 5 ml fractions were collected. The enzyme was well separated from the Dextran Blue at higher ionic strength and appeared in the inner volume as shown in Fig. 3. Active fractions from tubes 16 to 20 were pooled and dialysed against 0.001 M-sodium acetate buffer, pH 6. Both these column operations were carried out at 23°C.

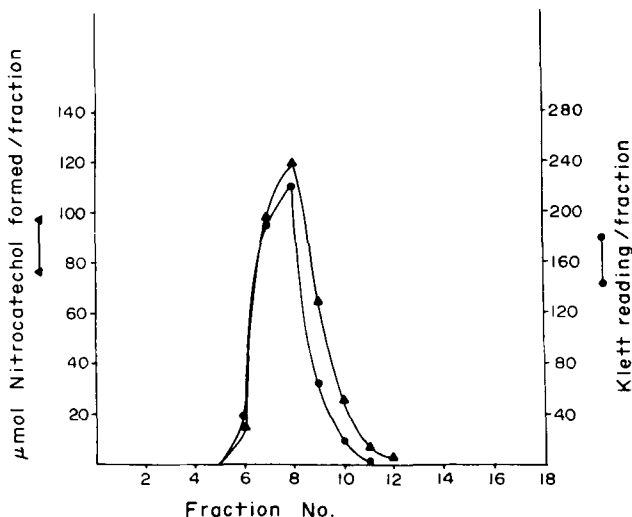


FIG. 2. Sephadex G-200 elution profile of arylsulphatase B-Dextran Blue complex. The column was equilibrated with 0.005 M-acetate buffer, pH 6. Fractions of 2 ml were collected and assayed the enzyme as described in Methods. (●) Klett reading with 66 filter for Dextran Blue. (▲) Enzyme activity.

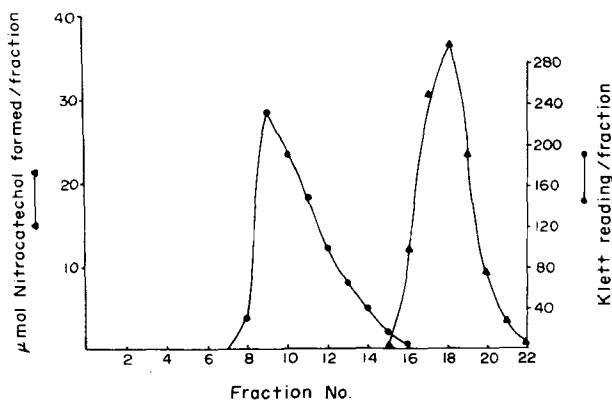


FIG. 3. Elution profile of dissociated arylsulphatase B-Dextran Blue on Sephadex G-200. The column was equilibrated with 0.02 M-acetate buffer, pH 6, containing 1 M-sodium chloride. Fractions (5 ml) were collected and assayed the enzyme as described in the Methods. (●) Klett reading, with 66 filter for Dextran Blue. (▲) Enzyme activity.

DEAE-cellulose chromatography. The enzyme solution from the previous step had 1 mg protein in 20 ml. To this 1 M-Tris-HCl buffer, pH 8, was added to a final concentration of 0.005 M and passed through a 2 ml column of DEAE cellulose at a flow rate of 15 ml/h previously equilibrated with the same buffer. The enzyme was completely adsorbed to the column and the column was washed with 10 bed volumes of the same buffer. The enzyme was eluted with a gradient formed from 20 ml of 0.005 M-Tris-HCl buffer, pH 8, and 20 ml of 0.5 M-sodium chloride, in 0.005 M-Tris-HCl buffer, pH 8. One millilitre fractions were collected at a rate of 0.3 ml/min. The active fractions eluted with buffer containing approx 0.1 M-NaCl were pooled and dialysed against 0.001 M-acetate buffer pH 6, and concentrated by dialysis against saturated ammonium sulphate. The precipitate formed was centrifuged and was dissolved in 0.01 M-acetate buffer, pH 6, and dialysed against the same buffer. This had 0.45 mg protein in 0.5 ml.

RESULTS AND DISCUSSION

The zinc acetate precipitate which contained mainly arylsulphatase B was used as a starting material for the purification of this enzyme. However, starting from homogenate the enzyme was purified 1344-fold. Since the assay does not differentiate the

two arylsulphatases, recovery was calculated from the zinc acetate precipitate which had mainly sulphatase B, and it was found to be 10.5%. The different steps involved in the purification are shown in Table 1. Reproducible results were obtained on the purification when this was repeated a number of times. Zinc acetate precipitate when adjusted to 0.5 M-acetate buffer, pH 5, resulted in precipitation of a large number of proteins. This step resulted in a 3-fold purification of the enzyme with a 65% recovery. Even this pH 5 supernatant fluid after exhaustive dialysis seem to contain some zinc ions which inhibited the enzyme activity and the correct measurement of the enzyme activity could not be done.

Earlier work from our laboratory has shown that brain lysosomal acid hydrolases are glycoproteins and they bind with concanavalin A (BISHAYEE & BACHHAWAT, 1974). It has also been shown that arylsulphatase A from sheep brain could be purified using this method (BALASUBRAMANIAN & BACHHAWAT, 1975). In the present study also this affinity chromatography method was used and by this single step the enzyme was purified 69-fold with 66% recovery. Since it is known that glycoprotein concanavalin A interaction

TABLE 1. PARTIAL PURIFICATION OF ARYLSULFATASE B FROM SHEEP BRAIN

Purification steps	Total volume (ml)	Total protein (g)	Total enzyme units	Specific activity	Fold purification	Yield* (%)
Homogenate	2950	82.6	7676	0.093	—	—
Alcohol supernatant fluid	1760	12.1	3297	0.24	2.6	—
Zinc acetate precipitate	204	4.48	571	0.127	1.3	100
pH 5 supernatant fluid	465	0.651	372	0.57	6.1	65
Concanavalin A-Sepharose affinity chromatography	25	0.0047	230	38	410	40
Binding and dissociation with Dextran Blue	20	0.001	120	116	1247	21
DEAE-cellulose chromatography	7	0.0005	60	125	1344	10.5

* The activity in zinc acetate precipitate was arbitrarily taken as the initial concentration of arylsulphatase B as discussed in the text.

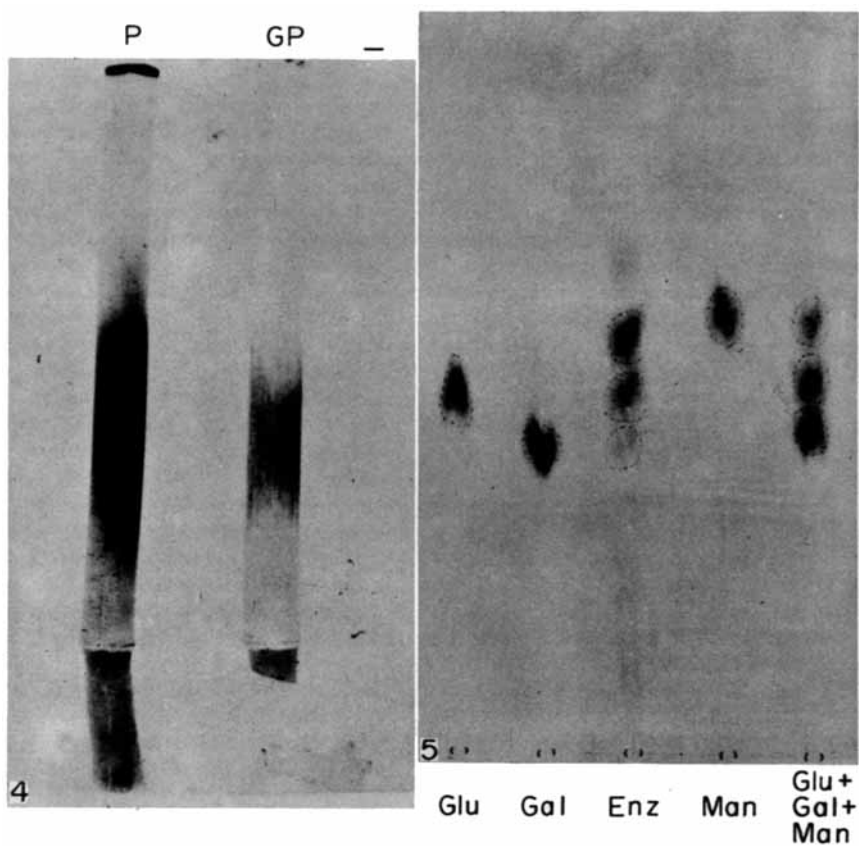


FIG. 4. Gel electrophoresis of arylsulphatase B at pH 4.5. The conditions for the electrophoresis were as described in the Methods. P and GP indicate the protein and glycoprotein stains respectively.

FIG. 5. Paper chromatographic pattern of neutral sugar of partially purified arylsulphatase B. The conditions of the experiment were as described in the text. ENZ indicates arylsulphatase B. Glucose, mannose, galactose and the mixture are indicated by Glu, Man, Gal and Glu + Gal + Man respectively.

is temperature dependent (BISHAYEE, 1973) and higher binding occurs at higher temperature, column operation was carried out at room temperature. By keeping the pH 5.0 solution at room temperature the enzyme did not lose its activity but some of the proteins were precipitated. These were removed by centrifugation and the clear supernatant fluid was used for passing through the column. The elution pattern of arylsulphatase B from concanavalin A-Sepharose column was quite interesting. It can be seen that the enzyme was not eluted with either alpha-methyl-D-glucoside or sodium chloride alone but it required both for elution. It is known that the concanavalin A-glycoprotein interaction involves not only sugar moieties, but also hydrophobic, ionic and protein-protein interactions (PODDER *et al.*, 1974). Thus with high ionic strength, i.e. in presence of 1M-sodium chloride this interaction was minimized and alpha-methyl-D-glucoside could elute the enzyme from the column. The requirement of sodium chloride in addition to methylglucoside has also been observed in the case of testicular hyaluronidase (BALASUBRAMANIAN *et al.*, 1975). It may be of interest to note that recently it has been observed by other investigators that venom exonuclease could not be eluted from the Sepharose-concanavalin A column by alpha-methyl-D-mannoside and the enzyme remained active and immobilized (SALKOWSKI & LUSKOWSKI, 1974). Similarly, interferon (another glycoprotein) was found to bind with the concanavalin A-Sepharose column and could be eluted with specific sugars in the presence of ethylene glycol and these authors conclude that apart from protein-carbohydrate interaction, some hydrophobic interactions also play an important role in the binding of this protein (DAVEY *et al.*, 1974). The binding of arylsulphatase B with concanavalin A and its elution with concanavalin A specific sugar suggested that sheep brain arylsulphatase B may be a glycoprotein.

It is known that depending on the ionic strength, Dextran Blue can bind with certain proteins (BLUME *et al.*, 1971; STAAL *et al.*, 1969, 1971; SWART & HEMKER, 1970). At low ionic strength Dextran Blue binds to the proteins and at high ionic strength it completely dissociates. The binding between protein and Dextran Blue may be ionic in nature and this property has been utilized for the purification of some enzymes. It was found that sheep brain arylsulphatase B also binds to Dextran Blue at low ionic strength. In 0.005 M it firmly bound to Dextran Blue and in first Sephadex G-200 gel filtration it appeared in the void volume together with Dextran Blue. But at high ionic strength this complex dissociated and in the second gel filtration it was very well separated from Dextran Blue and appeared in the inner volume. By this step a 3-fold purification was achieved with 50% recovery. It is known that unlike sulphatase A, sulphatase B has isoenzymes (ALLEN & ROY, 1968). Recently BLESZYNSKI & ROY (1973) separated as many as 7 isoenzymes from ox brain using ion exchange

chromatography. Sheep brain arylsulphatase B when passed through a DEAE cellulose column at pH 8 was adsorbed completely and could be eluted with a gradient of sodium chloride. However, in this case the enzyme eluted as a sharp peak providing no evidence for the presence of isoenzymes was obtained.

The purified enzyme when subjected to gel electrophoresis showed that all the regions which stained for protein and enzyme were also stained for glycoprotein (Fig. 4). This suggests that sheep brain arylsulphatase B is also a glycoprotein. The carbohydrate content of the enzyme was estimated and found to contain 11.7% neutral sugar and 0.4% sialic acid. The total neutral sugar content was less than half of that obtained for arylsulphatase A. As shown in Fig. 5 the neutral sugars present in the enzyme were glucose, mannose and trace amounts of galactose, however mannose was the predominant sugar. Thus it can be seen that like arylsulphatase A, arylsulphatase B also contains both glucose and mannose with the difference that the B form had in addition some galactose. This is quite interesting in that very few glycoproteins are known to contain glucose. However, the actual amount and the nature of the neutral sugar present in the enzyme has to be done with a homogenous preparation. Molecular weight determination of the enzyme was done at pH 6 in the presence of 1 M-NaCl. It was earlier noticed by ALLEN & ROY (1968) that there was a nonspecific binding of arylsulphatase B to the Sephadex column. The same property was also observed in the case of the sheep brain enzyme and 1 M-sodium chloride was used to equilibrate the column to prevent this nonspecific absorption. Molecular weight determined by gel filtration gave a value of 45,000 (Fig. 6). BLESZYNSKI & ROY (1973) found a mol wt of 45,000 by gel filtration and by sedimentation a value of 60,000 was observed for ox brain arylsulphatase B. They suggested that the discrepancy may be due to the nonspecific adsorption of sulphatase B to the Sephadex column.

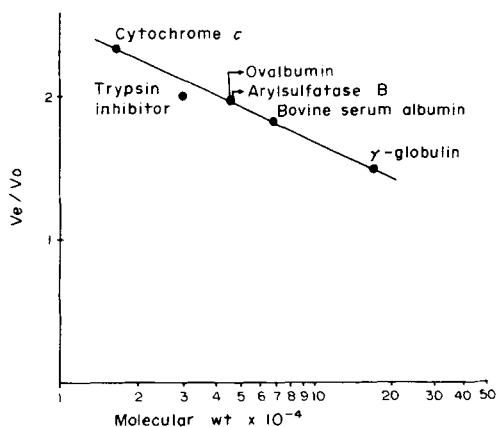


FIG. 6. The determination of molecular weight of arylsulphatase B by gel filtration on Sephadex G-200 at pH 6 in presence of 1 M-NaCl. The size of the column and other conditions are as described in the Methods.

Kinetic properties of arylsulphatase B. The enzyme had a pH optimum at pH 5.6 in sodium acetate buffer when incubated for 5 min at 37°C. A similar pH optimum was observed by ALLEN & ROY (1968) for ox liver arylsulphatase B whereas WOORWOOD *et al.* (1973) observed a pH optimum of 5.9 for the rat liver enzyme. HARINATH & ROBINS (1971) observed a pH optimum of 5.4 for the human brain arylsulphatase B with 4-methyl umbelliferone sulphate as substrate. It should be mentioned that the same pH optimum of 5.6 was observed for arylsulphatase A from the same source. K_m was determined with nitrocatechol sulphate as substrate, and with two independent experiments it was observed that sheep brain arylsulphatase B had K_m values of 0.375 mM and 0.45 mM. This was quite interesting since arylsulphatase B had a lower K_m than arylsulphatase A. HARINATH & ROBINS (1971) also observed that arylsulphatase B from human brain had a lower K_m than arylsulphatase A of the same tissue using 4-methylumbelliferone sulphate as substrate. This is in contrast to the reported results for ox liver enzyme. NICHOLLS & ROY (1971) have shown that ox liver arylsulphatase B had a much higher K_m than arylsulphatase A. It was observed that sheep brain arylsulphatase B had very little activity with *p*-nitrophenyl sulphate as substrate but a 6-fold activation was obtained in presence of 0.2 M-NaCl. This was similar to that reported for the ox liver enzyme (ALLEN & ROY, 1968). K_m was determined with *p*-nitrophenyl sulphate as substrate in presence of 0.2 M-sodium chloride which gave a value of 11 mM. The K_m value with *p*-nitrophenyl sulphate as substrate was lower for arylsulphatase B than for arylsulphatase A and this is similar to the reported value (ALLEN & ROY, 1968).

Sulphate, sulphite, phosphate and pyrophosphate were found to be inhibitory to this enzyme and the K_i values were determined. Sulphate and phosphate were found to be noncompetitive inhibitors whereas sulphite and pyrophosphate were competitive inhibitors. In contrast to this, arylsulphatase A was competitively inhibited by all these inhibitors. K_i value for pyrophosphate of arylsulphatase B was 10 times

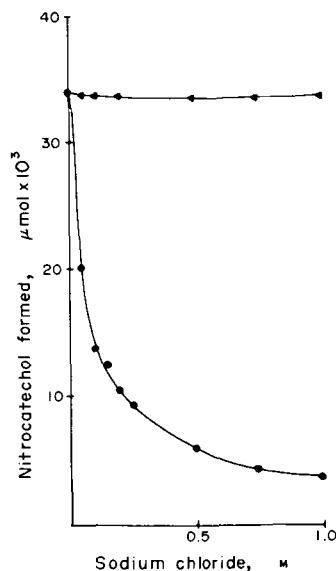


FIG. 7. Effect of sodium chloride on arylsulphatase A and B activity with *p*-nitrocatechol sulphate as substrate. Incubation mixture consisted of 0.25 M-acetate buffer, pH 5.6, 5 mM-nitrocatechol sulphate, 1.3 μg of arylsulphatase B or 0.05 μg arylsulphatase A and various concentrations of sodium chloride in a total volume of 0.4 ml. Incubation was at 37°C for 5 min and the reaction was stopped as described in the Method. (▲) Arylsulphatase A. (●) Arylsulphatase B.

lower than that of arylsulphatase A, similar to the observations of other investigators. The various kinetic parameters of arylsulphatase B and its comparison with arylsulphatase A are given in Table 2. Chloride was found to be an inhibitor for sheep brain arylsulphatase B when nitrocatechol sulphate was used as substrate. Even with 0.1 M-NaCl 50% inhibition was observed (Fig. 7). On the other hand arylsulphatase A was not affected by chloride and up to 1 M concentration no inhibition was observed. When *p*-nitrophenyl sulphate was used as substrate a 6-fold activation of arylsulphatase B activity was observed

TABLE 2. THE GENERAL PROPERTIES OF ARYLSULPHATASE A AND B FROM SHEEP BRAIN

	Sulphatase A	Sulphatase B
K_m (mM)		
<i>p</i> -Nitrocatechol sulphate	0.6	0.38
<i>p</i> -Nitrophenyl sulphate	45	11
pH optimum	5.6	5.6
K_i (M)		
Sulphate	0.35×10^{-2}	0.68×10^{-2}
Phosphate	0.18×10^{-4}	0.64×10^{-4}
Sulphite	0.7×10^{-5}	0.35×10^{-5}
Pyrophosphate	0.4×10^{-4}	0.35×10^{-5}
Effect of sodium chloride		
With <i>p</i> -nitrocatechol sulphate	No inhibition	Inhibition
With <i>p</i> -nitrophenyl sulphate	Weak action	6-fold activation
Molecular weight	122,000*	45,000

* Molecular weight determined at pH 7.5.

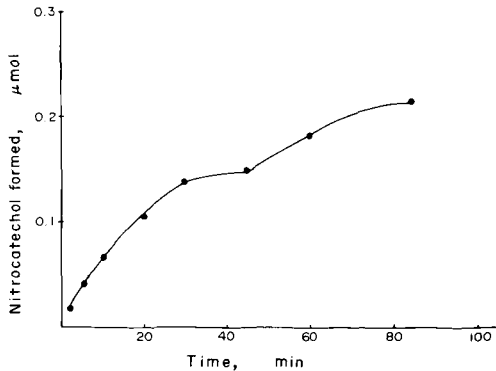


FIG. 8. Time activity curve of arylsulphatase B. The incubation mixture consisted of 0.25 M-acetate buffer, pH 5.6, 5 mM-nitrocatechol sulphate, 0.01% bovine serum albumin and 1.3 µg enzyme protein in a total volume of 0.4 ml. The reaction mixture was incubated at 37°C for various time intervals and the reaction was stopped as indicated in Methods.

whereas there was a very little activation of arylsulphatase A activity.

Another interesting feature of sheep brain arylsulphatase B was that it showed time anomalous kinetics (Fig. 8). Earlier observations on partially purified human arylsulphatase B indicated that this enzyme exhibited unusual kinetic anomalies in certain circumstances (DODGSON & WYNN, 1958). Similar anomalies were also apparent with partially purified ox liver enzyme but highly purified enzyme showed normal kinetics. The explanation given was that in the partially purified preparation the enzyme may combine with some inert protein and because of that it may show the anomalous kinetics. It has been clearly established that arylsulphatase A shows the anomalous kinetics but the highly purified preparation of sheep brain arylsulphatase B also showed the anomaly and it is not yet clear whether this is a peculiar property of sheep brain enzyme. Thus sheep brain arylsulphatase B resembles the enzyme from other sources in many, but not all, respects. A low K_m with nitrocatechol sulphate and the time anomalous kinetics are certain specific properties found in sheep brain arylsulphatase B and in this respect it differs from the arylsulphatase B of other tissues. With respect to molecular weight, activation by chloride when *p*-nitrophenyl sulphate was used as substrate and very low K_i for pyrophosphate, sheep brain arylsulphatase B was comparable to the arylsulphatase B of other tissues.

There has been no report on the glycoprotein nature of arylsulphatase B and this is the first study to show that arylsulphatase B is also a glycoprotein. Binding with concanavalin A, elution with concanavalin A-specific sugars, the glycoprotein nature of the purified protein as obtained on electrophoresis and the presence of neutral sugar in the partially purified enzyme clearly show that the sheep brain arylsulphatase B is a glycoprotein. The nature and actual

amount of neutral sugar present in the enzyme can be established only when a homogeneous preparation is obtained.

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