Biochimica et Biophysica Acta, 567 (1979) 184—195

BBA 68686

STUDIES ON THE CHAOTROPICALLY SOLUBILIZED ARYLSULFATASE C AND ESTRONE SULFATASE OF SHEEP BRAIN

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(Received September 14th, 1978)

Key words: Chaotropic agent; Arylsulfatase C; Estrone sulfatase; Solubilization

Summary

Arylsulfatase C (aryl-sulfate sulfohydrolase, EC 3.1.6.1) from sheep brain acetone powder was solubilized with the chaotropic agent, KSCN. Anti-chaotropes such as $(NH_4)_2SO_4$ or sodium citrate significantly enhanced the activity of the solubilized enzyme indicating that hydrophobicity was an important factor influencing the enzyme activity. Dialysis or gel filtration of the solubilized enzyme resulted in a marked loss of activity. A dialyzable activator could reconstitute the activity in the presence of the antichaotropes. The activator was purified partially and preliminary studies indicated it to be a low molecular weight peptide. Arylsulfatase C and estrone sulfatase activities were compared in the solubilized enzyme. Estrone sulfatase activity was also increased in the presence of antichaotropes at lower concentration in comparison to arylsulfatase C. It however did not show a requirement for the dialyzable activator. Kinetic studies showed that elevation of enzyme activity by the antichaotropes and activator in the case of arylsulfatase C and by antichaotropes in the case of estrone sulfatase was due to an increase in V with a decrease in K_m.

Introduction

Mammalian arylsulfatase C (aryl-sulfate sulfohydrolase, EC 3.1.6.1) differs from arylsulfatase A and B in its highly insoluble nature and alkaline pH optimum [1—3]. A close similarity between arylsulfatase C and estrone sulfatase activities in their subcellular localization, pH optima, thermal stability and inhibition by other steroid sulfates in the brain or liver has been observed while other steroid sulfatases showed properties different from that of aryl-

sulfatase C [1,4–6]. Hence the tentative conclusion that the physiological substrate for arylsulfatase C may be estrone sulfate has been arrived at. The presence of a pool of steroid sulfates and their sulfatases [7,8] in brain is significant and indicates the importance of the metabolism of steroid sulfates in brain. Furthermore a deficiency of arylsulfatase C and steroid sulfatases in the brains as well as liver of patients with multiple sulfatase deficiency has been demonstrated [9,10].

Studies on arylsulfatase C have been greatly hampered by the highly insoluble nature of the enzyme. Chaotropes are agents that interact with water structure resulting in the weakening of hydrophobic interactions and destabilization of biological macromolecules [11]. This property has been put to use for a variety of purposes involving destabilization of membrane components. Antichaotropes are water structure forming ions and tend to strengthen hydrophobic interactions. A list of chaotropes in the order of their potency and some commonly used antichaotropes have been given by Hatefi and Hanstein [12]. In the present work the enzyme arylsulfatase C from sheep brain has been solubilized by a chaotrope and the effect of antichaotropes on the solubilized enzyme has been studied. Experiments employing the physiological substrate, estrone sulfate, have also been performed to compare the behaviour of the two enzyme activities towards chaotropes and antichaotropes. A notable observation emerging from these studies is that hydrophobic bonds play an important role on enzyme activity. The results also indicate the requirement of a dialysable activator for the solubilized arylsulfatase C but not for estrone sulfatase. A partial characterization of the activator has also been made.

Materials and Methods

The potassium salt of *p*-nitrophenyl sulfate was prepared by the method of Milsom et al. [3]. The final product was crystallized six times from water. [6,9-^{3}H]Estrone sulfate (39 Ci/mmol) was purchased from Radiochemical Centre, Amersham. Salts like sodium citrate, $(NH_4)_2SO_4$, Na_2SO_4, NaF, sodium phosphate, KSCN, $NaClO_4$ were of the highest grade purity available. The pH of $(NH_4)_2SO_4$, Na_2SO_4, sodium citrate and sodium phosphate solution was adjusted to 8.0 before use as antichaotropes. The amino acids were purchased from either Calbiochem or Nutritional Biochemicals Corporation, U.S.A. Dipalmitoyl phosphatidylethanolamine, and cholesterol were products of Fluka, Switzerland and Centron Research, India, respectively. β-Mercaptoethanol was obtained from Mann Research, U.S.A.; sodium metaperiodate and trypsin from E. Merck, F.R.G.; D-α-dipalmitoyl phosphatidylcholine, glutathione and soybean trypsin inhibitor from Sigma Chemicals, U.S.A. Triton X-100 from Rohm and Haas; pronase (B grade) from Calbiochem, U.S.A. and Sephadex from Pharmacia, Sweden. Concanavalin-A was prepared according to the method of Surolia et al. [13]. Total lipids from fresh sheep brain were extracted by the method of Folch et al. [14].

Assay of aryl sulfatase C. The enzyme was assayed as described previously [6] based on the method of Milsom et al. [3]. The reaction mixture containing 20 μmol of potassium phosphate buffer, pH 8.0, 6 μmol of *p*-nitrophenyl sulfate and 0.05 ml of enzyme in total volume of 0.2 ml was incubated at 37° C

for 2 h at the end of which 2.5 ml ethanol and 0.5 ml of 1.0 M KOH were added, chilled in ice for 30 min, centrifuged and the colour intensity was measured in a Klett-Summerson Colorimeter using filter 42. Enzyme activity was linear with respect to time in all assays.

Assay of estrone sulfatase. The assay procedure was based on the method of Iwamori et al. [4]. The incubation mixture comprising 20 μmol of imidazole-HCl buffer, pH 8.0, [6,9-^{3}H]estrone sulfate ($9 \cdot 10^5$ cpm) and the enzyme was incubated at 37° C for 1 h. The reaction was arrested by adding 1.0 ml of 0.1 M Na_2CO_3. The liberated estrone was extracted thrice with 4.0 ml ether. The organic phase was transferred into a counting vial, evaporated to dryness and counted in a Beckman LS-100 Liquid Scintillation counter with a toluene/ethanol based scintillation fluid [15].

For substrate concentration-velocity studies the radioactive substrate was mixed with cold estrone sulfate such that the specific activity was 20 630 cpm/nmol. Reaction rates were linear with respect to time in all the assays.

Extraction of brain enzyme from acetone powder. Acetone powder of fresh sheep brain was prepared [16] and 24 g of the acetone powder was stirred for 1 h with 240 ml of 0.01 M potassium phosphate buffer, pH 7.5 containing 0.5 M of the chaotrope, KSCN and 0.025% β-mercaptoethanol. The mixture was homogenized in a potter-Elvehjem homogenizer and the homogenate was centrifuged at 105 000 × *g* for 60 min in a Beckman Model L ultracentrifuge. The supernatant was subjected to ammonium sulfate fractionation. The fraction precipitating at 30—60% saturation of $(NH_4)_2SO_4$ which gave the highest activity was dissolved in 0.01 M phosphate buffer, pH 7.5 to give a final volume of 9.0 ml and used for further studies. Extraction procedures were carried out at 0—4° C and the ammonium sulfate fraction was stored at —18° C when not required.

Purification of the dialyzable activator. In a typical procedure 9.0 ml of the ammonium sulfate fraction was dialyzed against 100 volumes of distilled deionized water for 6 h and the diffusate was concentrated to 3.0 ml by evaporation under vacuum at 30° C. The concentrated diffusate so obtained was subjected to polyacrylamide gel electrophoresis by the method of Davis [17] in 7% gels of length 4.2 cm using 0.02 M Tris-glycine buffer pH 8.0 at 6 mA per tube. In each tube 0.2 ml of the concentrated diffusate was applied. Soon after the tracking dy passed out the electrophoresis was stopped and the whole gel from each tube was crushed in 2.0 ml water and extracted for 48 h. The eluate was evaporated under vacuum to half the volume of the concentrated diffusate applied. This was again subjected to a second polyacrylamide gel electrophoresis as before. The gel after the run was sliced into 3 mm thickness and the slices assayed for the activator by addition to the incubation mixture. The gel slices containing the activator were subjected to extraction with water as before. The volume of the eluate after evaporation under vacuum was adjusted to half the original volume applied.

In a number of experiments the concentrated diffusate has been used as a source of the activator.

Proteolytic digestion. The concentrated diffusate was subjected to proteolytic digestion by trypsin and pronase at a concentration of 14.7 units/mg protein and 5.3 PUK units/mg protein of diffusate for 2 h and 24 h, respectively,

at 37° C. The reaction in the former case was stopped by adding 0.02 mg of trypsin inhibitor and in the latter by heating at 100° C for 5 min. Suitable controls were taken in each case.

Protein was estimated by the method of Lowry et al. [18] with crystalline bovine serum albumin as standard and ammonium sulfate was estimated as described by Chaney et al. [19].

Results

Solubilization of arylsulfatase C

Preliminary experiments done to solubilize the enzyme from sheep brain indicated that snake venom phospholipase treatment, limited proteolytic digestion or sonication did not result in any detectable release of enzyme into the 105 000 × *g* supernatant. The use of 1% (v/v) Triton X-100 gave only a pseudo-soluble preparation in the high speed supernatant. By treatment with the chaotrope, KSCN as described under methods a recovery of 12.6% from the homogenate was obtained in the 105 000 × *g* supernatant.

Effect of antichaotropes on ammonium sulfate fraction

Fig. 1 shows the effect of the antichaotropes $(NH_4)_2SO_4$, Na_2SO_4, sodium citrate, NaF and sodium phosphate at various concentrations on the solubilized arylsulfatase C. Addition of antichaotropes significantly raised the enzyme

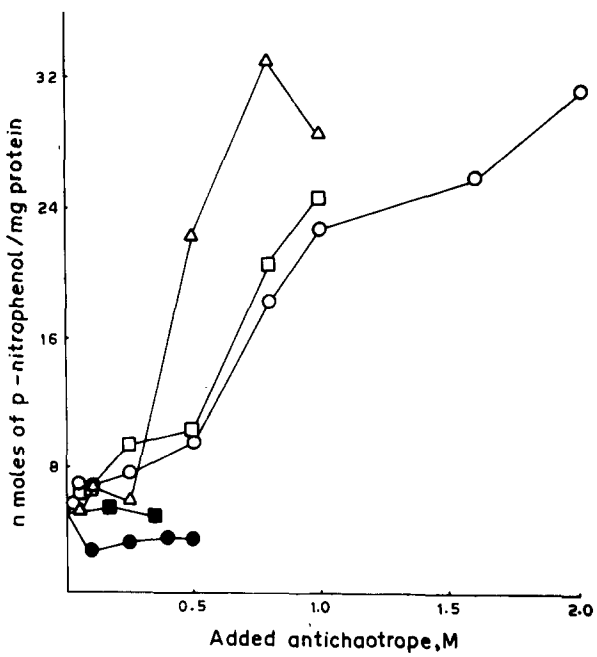

Fig. 1. Effect of antichaotropes on the arylsulfatase C activity of chaotropically solubilized sheep brain ammonium sulfate fraction. The antichaotropes at the indicated concentrations were added to the incubation mixtures. The concentration of ammonium sulfate originating from the brain enzyme in the reaction mixtures was 0.2 M. Substrate used in the incubation mixture was 2 μmol. ○———○, $(NH_4)_2SO_4$; □———□, Na_2SO_4; ■———■, NaF; △———△, citrate; ●———●, sodium phosphate.

activity. The order of antichaotropic potency was sodium citrate > $(NH_4)_2SO_4$ > Na_2SO_4 > sodium phosphate > NaF. The activity continued to increase up to 2.0 M $(NH_4)_2SO_4$ or 1.0 M Na_2SO_4 and a maximum activity was seen at 0.8 M citrate. It should be mentioned that $(NH_4)_2SO_4$ and citrate did not act like antichaotropes to enhance the arylsulfatase C activity when added at various concentrations to an acetone powder homogenate made in 0.02 M phosphate buffer, pH 7.5. In fact they were inhibitory to an extent of 29% and 35% at a concentration of 1.6 M and 0.8 M, respectively. The results indicated that the antichaotropes enhanced the arylsulfatase C activity only when added to the chaotropically solubilized enzyme.

Effect of chaotropes on brain arylsulfatase C containing the antichaotrope $(NH_4)_2SO_4$ or citrate

Fig. 2 shows the effect of addition of the chaotropes, KSCN and $NaClO_4$ to the solubilized brain enzyme containing the antichaotropes $(NH_4)_2SO_4$ or citrate. A significant decline in activity was observed at the higher concentrations of the chaotropes indicating the potency of chaotropes in reversing the effect of the antichaotropes. At lower concentration of the chaotropes however

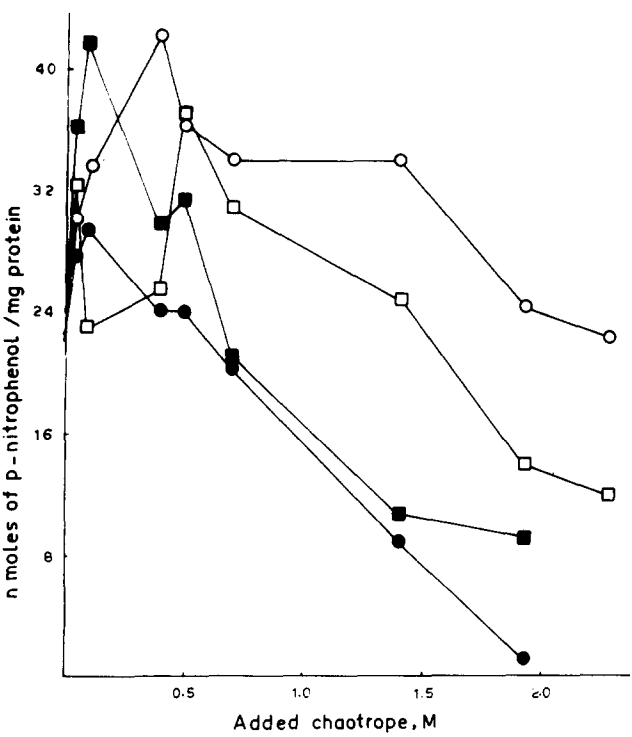

Fig. 2. Effect of chaotropes on brain arylsulfatase C: To the incubation mixture containing the brain ammonium sulfate fraction and the antichaotrope $(NH_4)_2SO_4$ (1.0 M) or citrate (0.5 M) The chaotropes were added at the indicated concentrations. Assay was as described under Fig. 1. ●———●, $(NH_4)_2SO_4$ as antichaotrope and KSCN as chaotrope; ○———○, $(NH_4)_2SO_4$ as antichaotrope and $NaClO_4$ as chaotrope; ■———■, citrate as antichaotrope and KSCN as chaotrope; □———□, citrate as antichaotrope and $NaClO_4$ as chaotrope.

there was either an increase or decrease in activity depending upon the concentration of the chaotrope added.

Loss of activity on dialysis of solubilized arylsulfatase C

Dialysis of the braine acetone powder homogenate in 0.01 M phosphate buffer pH 7.5, against 250 volumes of the same buffer for 6 h with 2 changes of buffer did not result in any loss of enzyme activity. However, dialysis under similar conditions or gel filtration on Sephadex G-200 of the solubilized ammonium sulfate fraction resulted in an activity loss of 93%. Only a slight increase in activity of the dialyzed enzyme was observed on addition of the antichaotropes $(NH_4)_2SO_4$ or citrate and the maximum activity attained at 1.0 M concentration of the antichaotropes was still at least 4–6 times lower when compared to the undialyzed enzyme under similar assay conditions.

Possible existence of a dialyzable activator

Experiments involving assays where allowances were made for the high concentration of $(NH_4)_2SO_4$ present in the concentrated diffusate suggested that there was an activator present in the diffusate and that the escape of $(NH_4)_2SO_4$ alone was not responsible for the loss in activity on dialysis. Thus addition of 1.6 M $(NH_4)_2SO_4$ alone gave a 3.5-fold activation of the dialyzed enzyme while the same concentration of $(NH_4)_2SO_4$ in the presence of the activator (0.05 ml of the concentrated diffusate) gave a 13.5-fold activation of the dialyzed enzyme (Table I). Similar results were observed when citrate was used in the presence and absence of the concentrated diffusate (Table I). Also included in Table I is an experiment in which the activator after purification by gel electrophoresis was used in the presence or absence of $(NH_4)_2SO_4$ for its effect on the dialyzed enzyme. It is seen that there was a 17-fold activation in

TABLE I

EFFECT OF THE CONCENTRATED DIFFUSATE OR THE PURIFIED ACTIVATOR AND THE ANTICHAOTROPES ON THE DIALYZED ARYLSULFATASE C

Incubation mixtures consisted of 6 μmol of *p*-nitrophenylsulfate, 20 μmol of potassium phosphate buffer, pH 8.0 and 0.05 ml of the dialyzed solubilized ammonium sulfate fraction. Assays were performed as given under Methods.

Expt.	Components added to the incubation mixture	Fold increase in activity over dialyzed enzyme
1	1.6 M $(NH_4)_2SO_4$	3.5
	0.8 M $(NH_4)_2SO_4$ + 0.05 ml concentrated diffusate (giving a concentration of 0.8 M $(NH_4)_2SO_4$)	13.5
	0.5 M sodium citrate + 0.8 M $(NH_4)_2SO_4$	4.0
	0.5 M sodium citrate + 0.05 ml concentrated diffusate (giving a concentration of 0.8 M $(NH_4)_2SO_4$)	24.5
2	0.05 ml eluate after first gel electrophoresis	2.0
	0.8 M $(NH_4)_2SO_4$	4.0
	0.05 ml eluate after first gel electrophoresis + 0.8 M $(NH_4)_2SO_4$	17.0

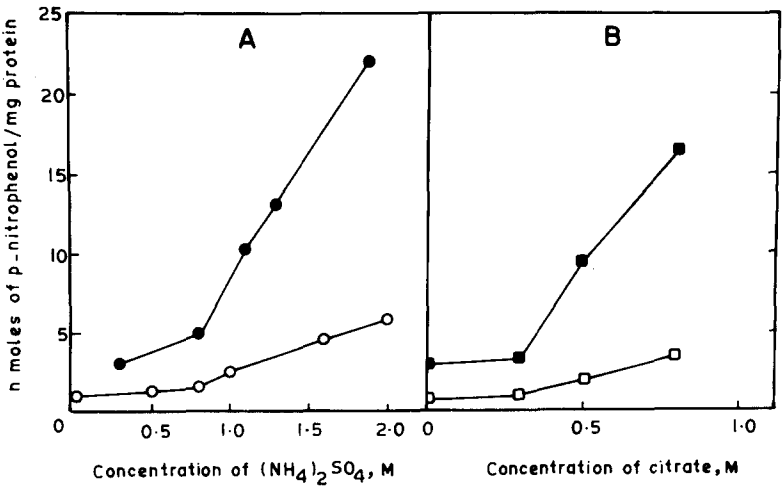

Fig. 3. Effect of the antichaotropes on the dialyzed arylsulfatase C in the presence and absence of the concentrated diffusate. Assay was as described under Fig. 1. 0.02 ml of concentrated diffusate (which when present in the incubation mixture gave a final concentration of 0.31 M $(NH_4)_2SO_4$ was used. (A) ○———○, $(NH_4)_2SO_4$; ●———●, $(NH_4)_2SO_4$ in the presence of concentrated diffusate (B) □———□, citrate + 0.31 M $(NH_4)_2SO_4$; ■———■, citrate in the presence of concentrated diffusate.

the presence of both 0.8 M $(NH_4)_2SO_4$ and the purified activator as compared to a 4-fold increase in the presence of 0.8 M $(NH_4)_2SO_4$ only or a 2-fold increase in the presence of the purified activator only. It appears from these results that the combined presence of both the antichaotrope as well as the activator is essential for the maximal activation of the dialyzed enzyme.

In Fig. 3 is shown the effect of the concentrated diffusate on the dialyzed enzyme in the presence of varying concentration of $(NH_4)_2SO_4$ or citrate. The significant enhancement of activity by the concentrated diffusate in the presence of $(NH_4)_2SO_4$ or citrate is evident from the figure.

The concentrated diffusate did not have any activating effect on an acetone powder homogenate made in 0.02 M phosphate buffer, pH 7.5. It also failed to enhance the activity of the undialyzed enzyme. Furthermore, the supernatant of the acetone powder homogenate heated at 100° C for 3 min did not activate the dialyzed enzyme in the presence of $(NH_4)_2SO_4$. These results suggested that the need for an activator arises only when the enzyme is treated with a chaotrope followed by dialysis and the activator could be a component of the membrane-bound enzyme, rendered dialyzable after chaotropic treatment.

Characterization of the activator

Preliminary studies showed that the activator present in the concentrated diffusate has a molecular weight below 5000 as observed by Sephadex G-25 gel filtration. It was thermostable. The activation effect was not lost after heating at 100° C for 3 min. It did not lose its activating effect after proteolytic digestion by trypsin or pronase as described under methods. Concanavalin A, a glycopeptide binding lectin failed to precipitate or inactivate the activator. Treatment with sodium metaperiodate [20] which results in the oxidation of carbohydrate moiety also did not inactivate the activator. Extraction of the

TABLE II

PURIFICATION OF THE ACTIVATOR OF ARYLSULFATASE C

Purification of the activator was carried out as described under Methods.

No.	Step	Volume (ml)	Total protein (mg)	Total units *	Specific units **	Fold purification
1	Concentrated diffusate	3.0	6.83	111.6	16.3	—
2	Eluate after gel electrophoresis I	1.5	4.06	80.0	19.7	1.2
3	Eluate after gel electrophoresis II	0.75	0.96	127.0	132.0	8.0

* One activator unit is defined as that amount of the activator peptide which increases the rate of *p*-nitrophenol liberated from *p*-nitrophenylsulfate by 100% under standard assay conditions using the dialyzed arylsulfatase C in the presence of 0.8 M $(NH_4)_2SO_4$ as antichaotrope.

** Specific units are units per mg protein.

diffusate with organic solvents such as chloroform, petroleum ether or ether did not remove the activator.

Phosphatidylcholine, phosphatidylethanolamine, cholesterol (1 mM) or a total lipid extract from sheep brain did not activate the dialyzed enzyme in the presence of antichaotropes. Also the amino acids Ala, Leu, Ile, Val, Pro, Phe, Try, Met, Lys, Arg, His, Glu, Cys, Gly, Thr (0.2 μmol each) or reduced glutathione (0.01 M) failed to activate the enzyme.

Table II shows the purification of the activator from the concentrated diffusate. The initial polyacrylamide gel electrophoresis mainly served as a method for desalting the activator as was evident by the sharp decrease in the $(NH_4)_2SO_4$ concentration from 2.8 M to 0.05 M. In the third step the activator was purified 8 times. The activator in the second gel electrophoresis was localized in gel slices 11 and 12. The eluate from second gel electrophoresis (60 μg protein) on two dimensional paper chromatography in the solvent system, phenol/0.2 M KCl-HCl buffer, pH 1.0 (50 : 7, v/v) for the first run and butanol/acetic acid/water (4 : 1 : 1, v/v/v) for the second run gave a single prominent spot when sprayed with 0.4% ninhydrin. Chromatography after hydrolysis of the eluate with 6 N HCl for 3 h at 110° C yielded at least four ninhydrin positive spots which were comparable in mobility to glutamic acid, cysteine, threonine and glycine.

Mode of action of antichaotropes and activator on arylsulfatase C

Kinetic studies were done to find out the mode of action of antichaotropes and the activator in enhancing the arylsulfatase C activity of the chaotropically solubilized enzyme. As shown in Table III (Expt. 1) V of the enzyme was significantly increased while the K_m was decreased in the presence of $(NH_4)_2SO_4$ or citrate. A similar observation was made with the dialyzed enzyme where the V was remarkably increased and the K_m decreased in the presence of both the activator and the antichaotropes (Table III, Expt. 2).

The antichaotrope $(NH_4)_2SO_4$ also seemed to enhance the thermal stability of the chaotropically solubilized arylsulfatase C. After preincubation at 60° C for 1 h, the undialyzed enzyme lost about 80% of its activity but in the presence of 1.6 M $(NH_4)_2SO_4$ the loss was only about 30%. Citrate at 0.8 M however did not have any significant effect on the thermal stability.

Studies with estrone sulfate as substrate

Since estrone sulfatase activity has been identified with arylsulfatase C activity [1,4–6] it was of interest to study the properties of the chaotropically solubilized brain enzyme with estrone sulfate as substrate. There was a demonstrable increase in estrone sulfatase activity by the addition of the antichaotropes $(NH_4)_2SO_4$ or citrate after dialysis of the enzyme. In Fig. 4 is shown the effect of $(NH_4)_2SO_4$ and citrate at varying concentrations on estrone sulfatase activity. The maximum activity was seen at 1.0 M $(NH_4)_2SO_4$ or at 0.8 M citrate concentrations which was lower than that necessary for the maximum activity of arylsulfatase C. A sharp drop in activity was observed at higher concentrations.

Dialysis of the enzyme although resulted in about 50% loss of estrone sulfatase activity this loss could be fully recovered by the addition of 1.0 M $(NH_4)_2SO_4$ or 0.8 M citrate as mentioned earlier. Therefore, it is presumable that the loss in estrone sulfatase activity on dialysis was purely due to the escape of the antichaotrope, $(NH_4)_2SO_4$. Furthermore addition of the concentrated diffusate or the purified activator did not enhance the estrone sulfatase activity of the dialyzed enzyme in the presence of the antichaotropes.

The effect of the antichaotropes $(NH_4)_2SO_4$ or citrate on K_m and V of estrone sulfatase is given in Table III, Expt. 3. Their mode of action on estrone

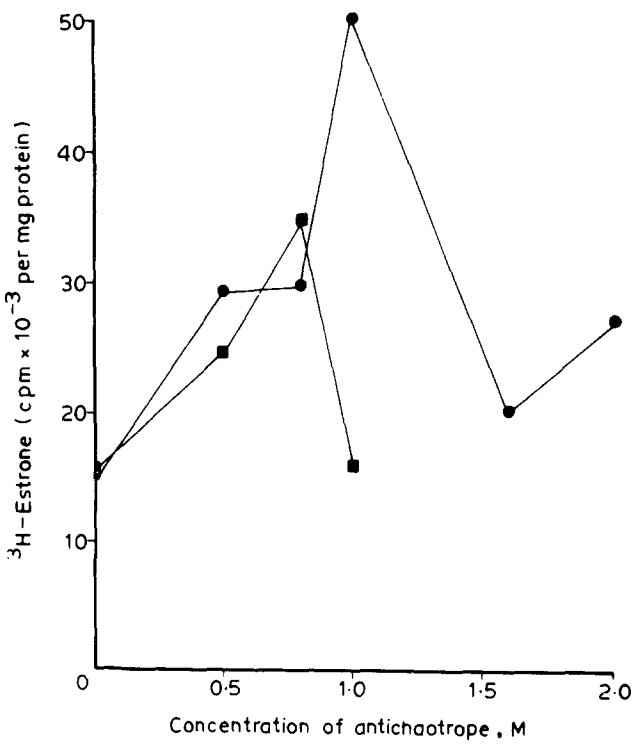

Fig. 4. Effect of antichaotropes on the estrone sulfatase activity of dialyzed brain arylsulfatase C. ●——●, $(NH_4)_2SO_4$; ■——■, citrate.

TABLE III

EFFECT OF ANTICHAOTROPES AND ACTIVATOR ON THE K_m AND V OF ARYLSULFATASE C AND ESTRONE SULFATASE

Expt.	Components *	K_m (mM)	V (nmol substrate hydrolyzed/mg protein per h)
1	Undialyzed arylsulfatase C	100	15.7
	Undialyzed arylsulfatase C + 1.6 M $(NH_4)_2SO_4$	50	31.4
	Undialyzed arylsulfatase C + 0.8 M citrate	66	31.4
2	Dialyzed arylsulfatase C + 1.2 M $(NH_4)_2SO_4$	200	10.1
	Dialyzed arylsulfatase C + 0.03 ml of concentrated diffusate in the presence of 1.2 M $(NH_4)_2SO_4$	25	20.1
	Dialyzed arylsulfatase C + 0.5 M citrate + 0.4 M $(NH_4)_2SO_4$	100	6.7
	Dialyzed arylsulfatase C + 0.5 M citrate + 0.03 ml of concentrated diffusate (0.4 M $(NH_4)_2SO_4$)	22	20.1
3	Dialyzed estrone sulfatase	0.5	1.2
	Dialyzed estrone sulfatase + 1.0 M $(NH_4)_2SO_4$	0.038	2.3
	Dialyzed estrone sulfatase + 0.8 M citrate	0.038	1.97

* Assays for arylsulfatase C were carried out in the presence of the components given in the table, 0.1 M potassium phosphate buffer pH 8.0 and *p*-nitrophenyl sulfate in the range of 2.5 mM to 50 mM (Expt. 1) and in the range of 15 mM to 50 mM (Expt. 2). Assays for estrone sulfatase were done in the presence of the components given in the table, 0.1 M imidazole-HCl buffer, pH 8.0 and estrone sulfate in the range of 0.05 mM to 0.2 mM (Expt. 3) K_m and V values were derived from Lineweaver-Burk plots.

sulfatase appears to be similar to that on arylsulfatase C in enhancing V values and in decreasing K_m values significantly.

Discussion

In the present work, arylsulfatase C of sheep brain has been solubilized using the chaotrope KSCN. Antichaotropes, particularly ammonium sulfate and citrate are able to enhance the activity of this chaotropically solubilized enzyme significantly. The action of the antichaotropes seems to be specific for the chaotropically solubilized enzyme since they do not enhance the activity of the acetone powder homogenate. It is obvious from these studies that hydrophobic bonds play an important role in the catalytic activity of arylsulfatase C. Treatment with chaotropes although solubilizes the enzyme results in a significant decrease in catalytic activity presumably due to the weakening of the hydrophobic interactions but in the presence of antichaotropes there is a strengthening of these hydrophobic interactions which results in a significant enhancement of catalytic activity. A drop in activity at the higher concentration of the antichaotrope, citrate (Fig. 1) as well as the irregular increase or decrease at the low concentrations of the chaotropes (Fig. 2) may be partly attributed to strong ionic interactions unfavourable to the catalytic activity of the enzyme. Another important feature of the chaotropic solubilization of arylsulfatase C is in rendering an essential factor for activity, possibly a membrane component, dialyzable. Both the dialyzable activator and the antichaotrope are needed for the maximal elevation of the activity of the dialyzed enzyme. The

following events may be presumed to occur. Treatment with the chaotrope leads to a destabilization and disruption of membrane components; the enzyme and the dialyzable membrane component essential for activity go into the 105 000 × *g* supernatant; in the presence of the antichaotrope there is a reconstitution tending to exhibit the original enzyme activity.

Preliminary studies on the activator of arysulfatase C indicate it to be a small molecular weight peptide. Although small molecular weight peptide activators of other hydrolytic enzymes are known [21—23] whether there is any relationship between them and the activator of arylsulfatase C is uncertain primarily because the activator of arylsulfatase C operates only in the presence of an antichaotrope with the dialyzed chaotropically solubilized enzyme.

The presence of the antichaotropes or the activator plus antichaotropes results in a definite increase in the V of arylsulfatase C with a decrease in K_m. This may be a direct result of the conferring of the required hydrophobicity and facilitating enzyme catalysis.

A comparison of the estrone sulfatase and arylsulfatase C activities in the sheep brain enzyme preparation shows certain similarities in their properties. For example, after chaotropic treatment both activities are enhanced in the presence of the antichaotropes $(NH_4)_2SO_4$ or citrate. However, the concentration of antichaotropes needed for optimum activity is low for estrone sulfatase as compared to arylsulfatase C. The mode of action of the antichaotropes on both enzyme activities appears to be the same i.e. by increasing the V of the reaction and decreasing the K_m. A striking difference, however, between the two activities is in the requirement of the activator by arylsulfatase C after dialysis but not by estrone sulfatase. The reason for this is not clearly understood particularly because of the different buffers used in assay. While phosphate buffer was obligatory for the assay of arylsulfatase C [3] we found that estrone sulfatase was markedly inhibited by phosphate (about 85% inhibition at 0.1 M concentration) in accordance with the findings of others [6] and therefore imidazole-HCl buffer was used [5,9]. Assuming that arylsulfatase C and estrone sulfatase activities are present in the same protein it may be concluded that the microenvironment around the binding sites for the two substrates is variant.

Acknowledgement

S.L. is a Junior Research Fellow of the Council of Scientific and Industrial Research, India.

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