

Arylsulfatase B of Human Lung

ISOLATION, CHARACTERIZATION, AND INTERACTION WITH SLOW-REACTING SUBSTANCE OF ANAPHYLAXIS

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ABSTRACT Arylsulfatase B was separated from arylsulfatase A in extracts of human lung tissue by anion exchange chromatography and further purified by gel filtration and cation exchange chromatography. Arylsulfatase B of human lung was similar to that enzyme in other tissues and species, exhibiting an apparent mol wt of approximately 60,000, a pH optimum for cleavage of 4-nitrocatechol sulfate (pNCS) of 5.5–6.0, and a sensitivity to inhibition by phosphate ions and especially pyrophosphate in the presence of NaCl. Human lung arylsulfatase B inactivated slow-reacting substance of anaphylaxis (SRS-A) in a linear time-dependent reaction in which the rate was determined by the enzyme-to-substrate ratio. Cleavage of pNCS by human lung arylsulfatase B was competitively suppressed by SRS-A. The finding that human lung tissue contains predominately arylsulfatase B discloses a potential regulatory mechanism for inactivation of SRS-A at or near the site of its generation.

INTRODUCTION

Slow-reacting substance of anaphylaxis (SRS-A)¹ is an approximately 500-mol wt, acidic, sulfur-containing (1, 2) mediator of immediate-type hypersensitivity reactions that is not stored preformed and is generated immediately before its release (3, 4). The tissue mast cell and the peripheral blood basophil appear to be sources of the mediator based upon SRS-A generation and release by IgE-dependent reactions (5–7). The finding that

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¹Abbreviations used in this paper: CM-52, carboxymethyl cellulose; DE-52, DEAE cellulose; pABS, *p*-acetylbenzenesulfonic acid; pNC, 4-nitrocatechol; pNCS, 4-nitrocatecholsulfate; SRS-A, slow-reacting substance of anaphylaxis.

arylsulfatases of limpet or mollusk origin inactivate SRS-A (2) led to the demonstration that arylsulfatase B isolated from human eosinophil polymorphonuclear leukocytes also inactivates SRS-A (8). The further knowledge that arylsulfatases are present in extracts of human lung tissue (9) and can be recognized in lamellar bodies of the type II pneumocyte of rabbit lung tissue (10) prompted the extraction and separation of arylsulfatase B from arylsulfatase A in human lung tissue. This in turn permitted the demonstration that arylsulfatase B from human lung tissue inactivates SRS-A.

METHODS

Atropine sulfate, 4-nitrocatechol sulfate (pNCS), 4-nitrocatechol (pNC) (Sigma Chemical Co., St. Louis, Mo.), human serum albumin, and ovalbumin five times recrystallized (Miles Laboratories, Inc., Miles Research Div., Elkhart, Ind.), blue dextran, Sephadex (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), *p*-acetylbenzenesulfonic acid (pABS), 4-hydroxyacetophenone (Eastman Kodak Co., Rochester, N. Y.), DEAE cellulose (DE-52), and carboxymethyl cellulose (CM-52) (Whatman Chemicals, Div. W. & R. Balston, Maidstone, Kent, England), Amberlite XAD-2 (100–200 mesh) and XAD-8, silicic acid (SilicAR CC7) (Mallinkrodt Chemical Works, St. Louis, Mo.), mepyramine maleate (Merck, Sharp & Dohme, West Point, Pa.), and histamine acid phosphate (Mann Research Laboratories, Inc., New York) were obtained as indicated. All solvents used in silicic acid chromatography were of Nanograde quality (Fisher Scientific Co., Pittsburgh, Pa.). Ragweed antigen E was provided by the Research Resources Branch, National Institute of Allergy and Infectious Disease, Bethesda, Md.

Purification of SRS-A

SRS-A was generated from the rat peritoneal cavity prepared with hyperimmune rat antiserum (11), or from human lung fragments sensitized with IgE (5) by antigen challenge, as previously described. The diffusates were adjusted

to 80% ethanol and centrifuged for 20 min at 15,000 *g* in a Sorvall RC-2B centrifuge (DuPont Instruments, Sorvall Operations, Newtown, Conn.) at 4°C; and the supernates were evaporated to dryness in vacuo at 50°C in a Buchler flash evaporator (Buchler Instruments Div., Searle Analytic, Inc., Fort Lee, N. J.). The SRS-A was resuspended in 4.5 ml distilled water, before the immediate addition of 0.5 ml 1 N NaOH, incubated for 30 min at 37°C, and applied to a 10 ml Amberlite column exhaustively washed with distilled water. The column was eluted with 30 ml water, followed by 30 ml 80% ethanol in water. The entire ethanol fraction was evaporated to dryness as above, resuspended in 1.0 ml absolute ethanol, and applied to a 5–10-ml silicic acid column equilibrated in hexane. The column was sequentially eluted with 30-ml volumes of hexane, dichloromethane, acetone, *n*-propanol, and ethanol:concentrated ammonia: water (vol/vol, 6:3:1) (1). The fractions were evaporated to dryness and resuspended in 1–2 ml distilled water before dilution with buffer appropriate to biochemical studies or bioassay on the atropinized antihistamine-treated guinea pig ileum (3, 12).

Lung arylsulfatase

Measurement. Arylsulfatase was quantitated by measurement of the cleavage product (pNC) generated from the synthetic substrate (pNCS) (13). Enzyme preparations, previously dialyzed against 0.5 M, pH 5.7, sodium acetate buffer and varying from 0.025 to 1.0 ml in volume, were incubated with 0.01 M pNCS in 2.0 ml of 0.5 M, pH 5.7, sodium acetate buffer for assessment of arylsulfatase B at 37°C for varying time periods, unless otherwise stated. The procedure was carried out in 0.5 M, pH 4.5, sodium acetate buffer for measurement of arylsulfatase A. The reactions were stopped by the addition of one-half the reaction volume of 1.0 N NaOH, and the quantity of pNC liberated was determined by measuring the OD₅₁₅ in a Gilford 300N spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). A standard curve was generated for each assay by the addition of base to graded amounts of pNC and measurement of the OD₅₁₅. Reagent blanks were made by incubating substrate for the requisite period of time and then adding base and enzyme simultaneously. One unit of enzyme activity is defined as that amount which liberates 1 μmol pNC/h from a concentration of 0.01 M pNCS in the standard buffer. The pH optimum of both arylsulfatases

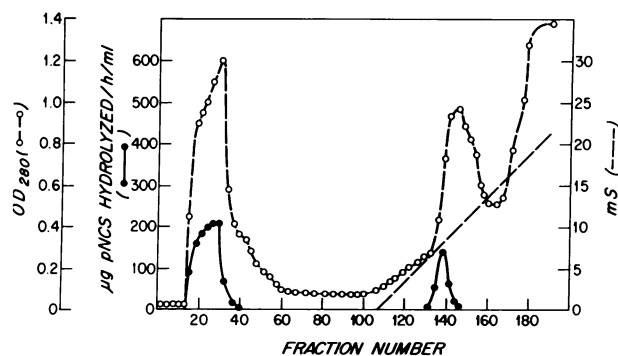


FIGURE 1 DE-52 cellulose anion exchange chromatography of arylsulfatase in human lung extract. Cleavage of pNCS at pH 5.7 and 4.5 is plotted for effluent and eluate, respectively.

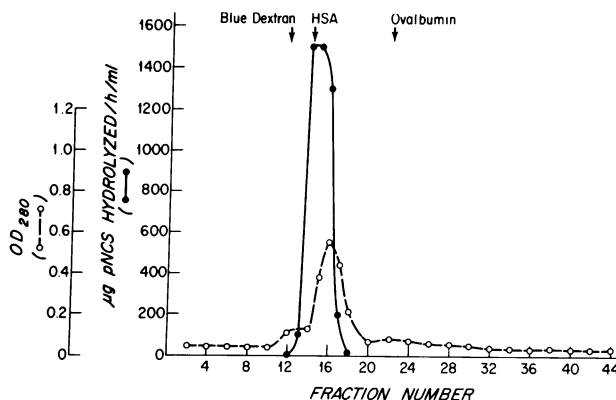


FIGURE 2 Sephadex G-75 gel filtration of the human lung arylsulfatase B present in the effluent obtained by DE-52 cellulose anion exchange chromatography. HSA, human serum albumin.

was measured by altering the ratio of sodium acetate to acetic acid in the 0.5 M acetate buffer made 0.01 M pNCS, and the substrate optimum for arylsulfatase B was assessed by altering pNCS concentration from 1 to 50 mM in the same buffer. V_{max} and apparent K_m were calculated from the linear plots of the reciprocal of the reaction velocity versus the reciprocal of the substrate concentrations, with 2.5–10 mM pNCS for 30 min at 37°C (14).

The hydrolysis of 0.01 M pABS was performed in 3 ml 0.5 M, pH 5.7, sodium acetate buffer for 60 min at 37°C. The reaction was stopped by the addition of 5 ml ethanol and 1 ml 1 N NaOH, and the degree of hydrolysis was assessed by measuring the OD_{327.5}. A standard curve was generated by the addition of 5 ml ethanol and 1 ml 1 N NaOH to graded amounts of 4-hydroxyacetophenone, and the OD_{327.5} was measured. Reagent blanks were made by incubating substrate for the requisite period of time and then adding ethanol, base, and enzyme simultaneously.

Isolation from lung. 50–200 g of human lung tissue obtained at the time of surgery were finely minced, washed free of blood in 0.15 M NaCl, homogenized in a Waring Blendor (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn.), sonicated (Ultrasonic Instruments International Ltd., Farmingdale, N. Y.), and centrifuged at 15,000 *g* for 15 min at 4°C. The supernate was dialyzed against 20 vol of 0.01 M Tris-HCl buffer, pH 8.0, overnight at 4°C with one change, and applied to a 2.5 × 20-cm DE-52 cellulose column equilibrated in the same buffer. The column was washed with three bed volumes of the equilibrating buffer and eluted with an 800-ml linear salt gradient to 0.4 M NaCl at 35 ml/h. Fractions of 7.5 ml each were collected, and 0.05–0.2 ml of alternate fractions was assessed for arylsulfatase activity. The effluent and eluate fractions containing arylsulfatase activity were concentrated to 3 ml each by ultrafiltration (Amicon Corp., Scientific Systems Division, Lexington, Mass.). The effluent arylsulfatase was applied to a 1.5 × 80-cm Sephadex G-75 column and the eluate arylsulfatase to a Sephadex G-200 column of the same dimensions, each in 0.01 M Tris-HCl buffer, pH 8.0, made 0.10 M NaCl. The columns were developed at 10 ml/h, 2.5-ml fractions were collected, and 0.025–0.10-ml portions were assessed for arylsulfatase activity. The fractions containing the peak of activity from the Sephadex G-75 column were pooled, dialyzed overnight at

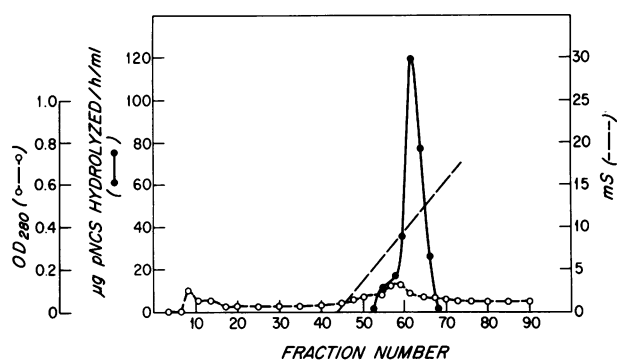


FIGURE 3 CM-52 cellulose cation exchange chromatography of human lung arylsulfatase B from DE-52 and Sephadex G-75 chromatography.

4°C against 0.05 M, pH 5.0, sodium acetate buffer with one change, and applied to a 1 × 20-cm CM-52 column equilibrated in the same buffer. The column was washed with six bed volumes of equilibrating buffer and eluted with a linear salt gradient of 300 ml to 0.3 M NaCl, at 8 ml/h. 8-ml fractions were collected and 0.05–0.20 ml of each was assessed for arylsulfatase activity. The fractions containing the peak of activity were pooled and concentrated to 6 ml for analytical acid and alkaline polyacrylamide gel electrophoresis, biochemical characterization, and interaction with SRS-A. Protein was estimated by absorbance at OD₂₈₀.

Alkaline disc gel electrophoresis was performed in 7.5% polyacrylamide gels with 2.5% polyacrylamide stacking gels, and acid disc gel electrophoresis was carried out in 6.0% polyacrylamide gels, in both instances by the instructions supplied by the manufacturer (Canalco, Inc., Rockville, Md.). Replicate gels were subjected to three different procedures: denaturation in trichloroacetic acid and staining with coomassie blue dye for protein; direct visualization for arylsulfatase activity by incubation of the entire gel in 3.0 ml 0.01 M pNCS in 0.5 M, pH 5.7, sodium acetate buffer for 3 or 15 min, followed by addition of 1.5 ml 1 N NaOH for 15 min at 37°C and washing of the gel (15); and division into 3-mm segments and elution for 18 h at 4°C in 0.2 ml of 0.5 M, pH 5.7, sodium acetate buffer, followed

TABLE I
Purification of Human Lung Arylsulfatase

Preparation	Total protein mg	Enzyme activity		Specific activity U/mg protein
		pH	μg pNCS hydrolyzed/h	
Starting material	1,300	4.5	17,000	0.042
		5.7	25,250	0.062
DE-52 effluent	115	4.5	3,642	0.103
		5.7	17,050	0.463
DE-52 eluate	68	4.5	4,990	0.238
		5.7	1,425	0.068
Sephadex G-75 (DE-52 effluent)	62	4.5	2,303	0.119
		5.7	11,250	0.582
CM-52 (G-75 peak)	2.3	4.5	1,275	0.177
		5.7	7,540	10.540

by addition of the standard reaction mixture directly to the tube and removal of particulate material by centrifugation before measurement of the OD₆₁₅ to assess arylsulfatase activity.

Interaction with SRS-A. Reaction mixtures of 2.0–4.0 ml volume to allow repeated sampling for bioassay, containing SRS-A of human or rat origin and arylsulfatase in amounts noted for the experiments, were incubated in 0.2 M, pH 5.7, sodium acetate buffer at 37°C for varying periods of time. Portions were removed from the reaction mixtures and diluted in Tyrode's buffer for bioassay of residual SRS-A; when necessary, the pH was adjusted to 7.8 by addition of 0.01 N NaOH before bioassay. SRS-A preparations were incubated alone and assayed in parallel; the arylsulfatase preparations had no gut-contracting activity. The effect of SRS-A upon arylsulfatase hydrolysis of pNCS was assessed by adding varying amounts of SRS-A of rat origin, purified through silicic acid, to 2.0-ml reaction mixtures containing 2.5–15 mM pNCS. The reaction mixtures were then incubated for 30 min at 37°C. Reagent blanks were obtained by incubation of substrate and SRS-A for the appropriate interval, followed by the simultaneous addition of enzyme and base.

RESULTS

Isolation of arylsulfatase B from human lung tissue. The arylsulfatase activity in the initial extracts from six different human lung preparations ranged from 25–85 U/g of extract protein, representing a content of 0.5–1.4 U/g wet lung tissue. DE-52 cellulose anion exchange chromatography of the extract yielded a predominant peak of activity in the effluent and another in the eluate at 6–8 mS (Fig. 1). The arylsulfatase activity in the effluent fractions 15–32 filtered on Sephadex G-75 in a single peak (Fig. 2), overlapping human serum albumin and preceding ovalbumin, compatible with a mol wt of approximately 60,000, as previously observed for arylsulfatase B of ox brain (16) and human eosinophils (8). The arylsulfatase activity of the eluate fractions 135–145 filtered on Sephadex G-200, which had been calibrated with dextran blue, aldolase, human serum albumin, and ovalbumin, between aldolase and human serum albumin, indicating an apparent mol wt

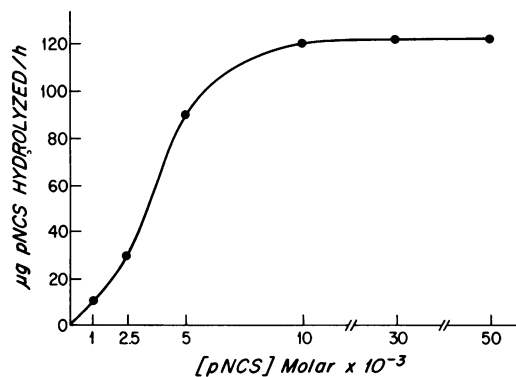


FIGURE 4 Effect of pNCS concentration upon hydrolysis by purified human lung arylsulfatase B.

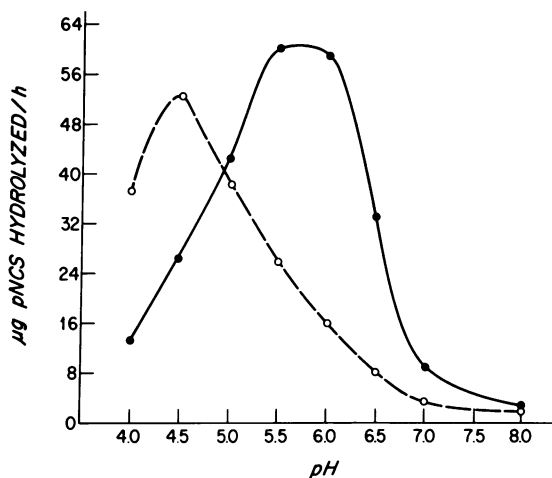


FIGURE 5 Effect of pH upon hydrolysis of pNCS by purified human lung arylsulfatase (●—●, arylsulfatase B; ○—○, arylsulfatase A).

of 100,000. This molecular weight is consistent with that previously observed for arylsulfatase A of human brain (17), ox liver (18), and human urine (19). The arylsulfatase B filtering in fractions 13–17 from Sephadex G-75 was pooled and subjected to CM-52 cellulose cation exchange chromatography (Fig. 3), from which it eluted as a single peak at 8–12 mS. The specific activity of the arylsulfatase B purified by anion and cation exchange chromatography and gel filtration was 10.5 U/mg protein (Table I). Two other preparations purified in the same fashion contained 7.3 and 9.2 U/mg protein, respectively. 0.4 U of this material was analyzed by alkaline and acid disc gel electrophoresis. A single band staining for protein was noted on alkaline disc gel electrophoresis, but enzyme activity was not eluted from any region of replicate unstained gels. In acid disc gel electrophoresis, two bands stained for protein, with activity being eluted from replicate unstained gels in a region superimposable upon the more cathodal, less densely staining band. Arylsulfatase functional activity, migrating in the identical region of parallel acid disc gels, was also demonstrated directly by generation of a colorimetric product by substrate cleavage, with unsliced gels.

Functional characterization of arylsulfatase B purified from human lung tissue. 0.4 U of purified arylsulfatase B (Figs. 1–3) was interacted with substrate concentrations varying from 1 to 50 mM pNCS for 60 min at 37°C. The quantity of pNCS hydrolyzed was maximal when concentrations of 0.01–0.05 M were employed, although the percentage of available substrate actually

being hydrolyzed diminished with increasing substrate concentration beyond 5 mM (Fig. 4). To define the pH optimum, 0.2 U of purified arylsulfatase B was interacted with 0.01 M pNCS for 60 min at 37°C at varying pH levels. The pH optimum was in the range of 5.5–6.0, with activity being markedly depressed at pH 4.5 (Fig. 5). The pH optimum for arylsulfatase A partially purified by elution from DE-52 and Sephadex G-200 gel filtration was 4.5, as determined under the same reaction conditions employed for arylsulfatase B. pNCS cleavage was comparable for the two enzyme preparations at their respective pH optima. Purified arylsulfatase B, exhibiting 0.1 U of activity with regard to pNCS, exhibited only 0.022 U when pABS was the substrate, as in the findings with arylsulfatase B from other tissues (8).

The V_{max} and apparent K_m of purified arylsulfatase B were estimated by interacting 0.33 and 0.67 U with varying substrate concentrations for 30 min at 37°C. The apparent K_m , as estimated directly from the intersection with the abscissa, was 1.5 mM. The V_{max} , estimated directly from the intersection with the ordinate, was 3,737 µg pNCS hydrolyzed/h/mg protein.

The effect of the introduction or deletion of ions from the standard reaction mixture was analyzed with 0.1 U of purified arylsulfatase B and an interaction time of 60 min at 37°C. Arylsulfatase B was exquisitely sensitive to phosphate ion, showing dose-related inhibition by 1–10 mM phosphate (Table II). The concentrations of Na_2SO_4 required to achieve comparable inhibition were 100-fold those of phosphate. Complete inhibition was achieved with 0.25 mM pyrophosphate ion in the presence of 1 M NaCl, a finding that distinguishes arylsulfatase B from arylsulfatase A (20). Neither the in-

TABLE II
Inhibition of Human Lung Arylsulfatase B

Ion	Concentration	Enzyme activity	Inhibition
	µM	µg pNCS hydrolyzed/h	
None		32	
Phosphate	10	4	88
	3	12	62
	1	17	47
Sulfate	100	18	44
	30	22	31
	10	28	12
Barium	50	29	9
	10	31	3
EDTA	10	31	3
	1	32	0
1 M NaCl + pyrophosphate	0.25	0	100

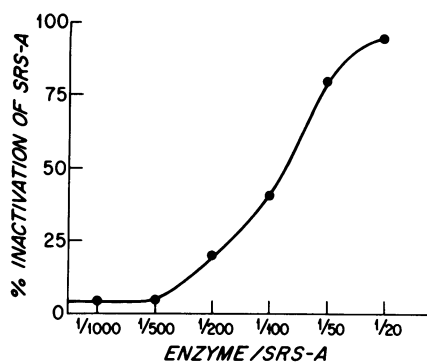


FIGURE 6 Inactivation of 100 U rat SRS-A by varying amounts of purified human lung arylsulfatase B. The abscissa expresses the ratio of arylsulfatase B to SRS-A in the arbitrary units for each.

roduction of barium to precipitate sulfate, a maneuver that facilitates arylsulfatase A activity, nor the presence of EDTA influenced the reaction.

Interaction of SRS-A with arylsulfatase B purified from human lung. The interaction of purified arylsulfatase B with purified SRS-A was examined at 37°C for 90 min with enzyme substrate ratios ranging from 1/1,000 to 1/20, calculated on the respective unit basis for each component. The reaction mixture contained 100 U of SRS-A and 0.1–5.0 U arylsulfatase B, in sodium acetate buffer at a concentration of 0.2 M rather than at 0.5 M to eliminate the effect of ionicity on the subsequent bioassay for SRS-A. Nearly complete inactivation of rat SRS-A was achieved by the 1/20 ratio, with 1/200 being the threshold ratio for an effect (Fig. 6). A 1/20 ratio also achieved complete inactivation of 80 U of human SRS-A under the same conditions. Ratios of 1/50 and 1/100 were selected to examine the kinetics of inactivation of 80 U of rat SRS-A over 3½ h at 37°C in 0.2 M sodium acetate buffer. The inactivation was linear at both enzyme concentrations, reaching com-

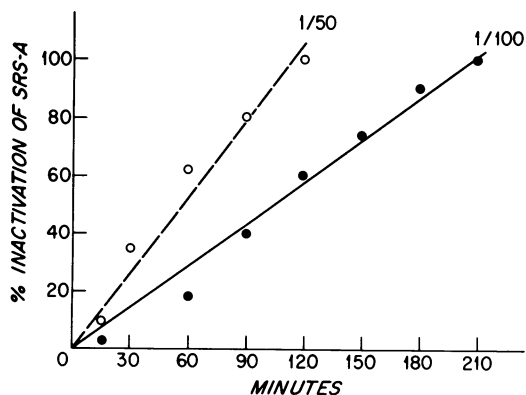


FIGURE 7 Time-course of inactivation of 80 U of rat SRS-A by 0.8 and 1.6 U of purified human lung arylsulfatase B.

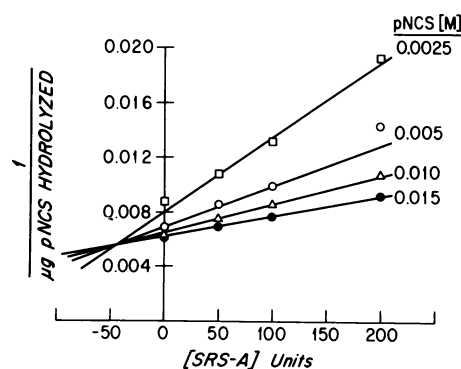


FIGURE 8 Effect of rat SRS-A upon the hydrolysis of pNCS by purified human lung arylsulfatase B.

pletion in 2 h at the 1/50 ratio and in 3.5 h at the 1/100 ratio (Fig. 7).

To determine if the same site in the purified arylsulfatase B was involved in the cleavage of pNCS and in the inactivation of SRS-A, a competitive experiment was performed. To each standard reaction mixture containing 2.5, 5.0, 10, or 15 mM pNCS were added 0, 50, 100, and 200 U rat SRS-A. After preincubation at 37°C, 1.0 U of purified arylsulfatase B from human lung was added. After a 30-min incubation at 37°C, the reactions were stopped and analyzed in the usual fashion. Plots of the reciprocal of the reaction velocity versus inhibitor concentration intersected above the abscissa and to the left of the ordinate (Fig. 8), consistent with a competitive inhibition (21).

DISCUSSION

The finding that arylsulfatase B of human lung tissue is capable of inactivating SRS-A of either rat or human origin reveals a mechanism by which the concentration of a mediator of immediate-type hypersensitivity generated and released by lung tissue may be modulated. The arylsulfatase B extracted from human lung chromatographed as a single peak sequentially on DEAE cellulose (Fig. 1), Sephadex G-75 (Fig. 2), and CM cellulose (Fig. 3). The specific activity of three different preparations ranged from 7.3 to 10.5 U/mg, and such preparations exhibited at least one contaminant as assessed by both alkaline and acid disc gel electrophoresis. The arylsulfatase activity in the initial lung extract was predominately arylsulfatase B, based upon the greater pNCS cleavage at pH 5.7 as compared to that at 4.5 (Table I). This view is supported by the finding of approximately three times the arylsulfatase B activity in the effluent as compared to that of arylsulfatase A in the eluate on the initial DE-52 cellulose chromatogram, when compared at their respective pH optima (Fig. 1, Table I). Previous studies with sperm (22),

human brain (17), and ox or beef cornea (23) also demonstrated that arylsulfatase B appeared in the effluent and arylsulfatase A in the eluate on DEAE chromatography, but differed in that the predominant species was arylsulfatase A. If approximately three-quarters of the arylsulfatase activity in the initial lung extract was B, the overall recovery of arylsulfatase B was approximately 40%. The lung arylsulfatase B resembled the enzyme isolated by others from cornea (23), liver (18), brain (16, 17), and eosinophils (8) in exhibiting a mol wt of approximately 60,000 (Fig. 2), a pH optimum between 5.5 and 6.0 (Fig. 5), and an exquisite sensitivity to inhibition by phosphate (8, 17) or pyrophosphate in the presence of 1 M NaCl (Table II) (20). The apparent K_m of 1.5 mM for pNCS is similar to that of the eosinophil and limpet enzymes (8), as well as arylsulfatase B isolated from other tissues and species (17, 24).

The capacity of human lung arylsulfatase B to inactivate SRS-A is dose-related (Fig. 6) and time-dependent (Fig. 7). The time-dependent inactivation is linear, with complete inactivation achieved at different rates, depending upon enzyme substrate ratios (Fig. 7). That the inactivation of SRS-A by arylsulfatase B represented intrinsic arylsulfatase activity of the enzyme is indicated by the capacity of SRS-A to suppress pNCS cleavage competitively (Fig. 8). Such an interaction was previously also observed with human eosinophil arylsulfatase B (8).

The eosinophil is recruited to the site of an immediate-type hypersensitivity reaction by the performed mast cell-associated (25) tetrapeptide (26) mediator, eosinophil chemotactic factor of anaphylaxis (27), and is assumed to inactivate SRS-A, another mediator generated by the mast cell reaction. The eosinophil contains only arylsulfatase B, and SRS-A inactivation is achieved through interaction with either the isolated enzyme (8) or the intact eosinophil (28). The expression of this control mechanism obviously requires tissue infiltration with this cell type. In contrast, the finding that human lung tissue contains predominantly arylsulfatase B as compared to A uncovers an inherent regulatory mechanism by which SRS-A could be inactivated at, or near, the site of its generation.

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