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# Isolation and Purification of Human Pulmonary Arylsulfatase B by Means of Chromatofocusing

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Arylsulfatase B (AS-B) activity was isolated from the human lung. AS-B activity has been reported to be homogeneous on disc electrophoresis after ion-exchange chromatography, but in the present study, a highly purified (approx. 2000-fold increase as compared with the previously reported 170-fold increase of specific activity) fraction was found to be still heterogeneous on a chromatofocusing column.

**Keywords**——arylsulfatase B; arylsulfatase; human lung; chromatofocusing; arylsulfatase B heterogeneity; 4-methylumbelliferyl sulfate

It is well known that there are two arylsulfatase activities [EC 3.1.6.1 arylsulfate sulfohydrolase] (AS), that is, AS-A and AS-B, in the lysosomal fraction.[1] AS-A has been well characterized in terms of enzymatic properties, isolation, purification and kinetics, but little is known about AS-B in the lung.

In the present study, the authors examined the isolation and purification of human lung AS-B by means of a chromatographic technique on the basis of previous fundamental studies on the determination of AS activity, using 4-methylumbelliferyl sulfate as a substrate, with high sensitivity.[2,3]

## Materials and Methods

Human lung was excised at autopsy within several hours after death from a subject having no disease in the

TABLE I. Purification of Human Lung AS-B

| Step | Procedure | Purification (fold) | | | |
|---|---|---|---|---|---|
| | | Wasserman's method | Method I | Method II | Method III |
| 1 | Starting material | 1 | 1 | 1 | 1 |
| 2 | $(NH_4)_2SO_4$ precipitation | — | — | 2.6 | 2.6 |
| 3 | DE-52 ion-exchange chromatography column method | 7.5 | 71.4 | 257.4 | — |
| 3′ | DE-52 ion-exchange chromatography batch method | — | — | — | 23.3 |
| 4 | Sephadex G-75 gel-filtration | 9.4 | 106.2 | 286.5 | 647.9 |
| 5 | CM-52 ion-exchange chromatography | 170.0 | — | 684.3 | 901.1 |
| 6 | PBE 118 chromatofocusing | — | — | — | 1993.7 |

Numbers indicate purification (fold) based on the specific activities.

respiratory organs. Isolation and purification of human pulmonary AS were performed according to a modified method described by Wasserman and Austin,[4] that is, 40—80% (w/v) solid $(NH_4)_2SO_4$ was added to an aliquot of the lung homogenate. The obtained precipitate was dialyzed overnight against 0.005 M Tris-HCl buffer, pH 8.0, and then the dialysate was subjected to column chromatography or batch adsorption on DEAE-cellulose (DE-52, Whatman Ltd.) previously equilibrated with 0.01 M Tris-HCl buffer, pH 8.0. The non-adsorbed fraction on DE-52 (AS-B fraction) was subjected to ultrafiltration on an Amicon PM-10 membrane (Amicon Far East Ltd.), and the concentrate was passed through Sephadex G-75 (Pharmacia Fine Chem.) (gel filtration) and CM-52 (Whatman Ltd.) (ion-exchange) columns.

The purified AS-B fraction obtained by the above procedure was subjected to chromatofocusing (CF) on PBE 118 (Pharmacia Fine Chem.) equilibrated with 0.0125 M dimethylglycine Na·HCl buffer, pH 10.5, or with 0.025 M triethylamine·HCl buffer, pH 10.5, and a linear pH gradient, from pH 10.5 to 8.0, was applied with the eluting buffer, Pharmalyte-HCl buffer, pH 8.0.

As the activity was determined by a modification of the method[5] of Christomanou and Sandoff,[6] protein was assayed with Coomassie Brilliant Blue R-250 (Bio Rad Lab.). Since Wasserman and Austin[4] determined the amount of protein by Lowry's method, the correlation between the two methods of protein assay was examined in connection with the specific activity of the enzyme, and the specific activity was expressed in the present study using the protein amount determined by the use of Coomassie Brilliant Blue R-250.

## Results and Discussion

Wasserman and Austin[4] reported that human pulmonary AS-B was purified 170-fold by CM-52 ion-exchange chromatography, and claimed that the fraction thus obtained was homogeneous on disc electrophoresis.

In the present study, the purification of AS-B of human lung was successfully performed by ammonium sulfate fractionation, followed by a series of column chromatographies, *i.e.*, DE-52 (ion-exchange); column or batch method (see ref. 5), Sephadex G-75 (gel filtration) (Fig. 1), and CM-52 (ion exchange) (Fig. 2), and finally chromatofocusing on PBE 118 (Fig. 3). This procedure provided about 2000-fold purification in terms of specific activity, and the final step (chromatofocusing) gave three fractions with AS activity. The specific activity of the first AS fraction on chromatofocusing was found to be increased 1890-fold from the starting material. The peak shape of the final peak suggests that it may still contain impurities.

The optimal pH of AS-B was at pH 5.5 when determined in 0.5 M acetate buffers (pH 4.5, 5.0, 5.5 and 6.0) containing 10 mM 4-MU sulfate as the substrate (see ref. 5). However, the three AS-B fractions were eluted at pH 9.42, 9.35 and 9.06 from the chromatofocusing column, and were extremely unstable, unless the pH of the eluates was immediately adjusted

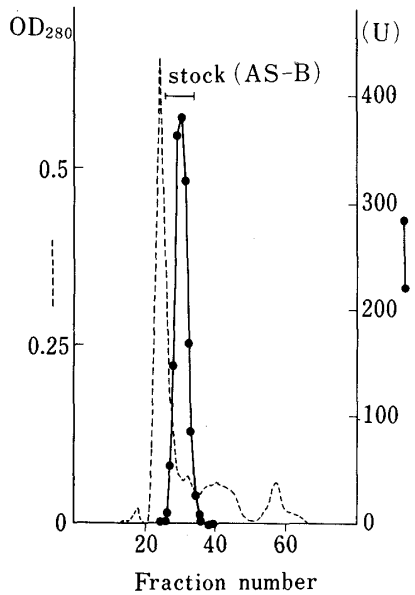


Fig. 1. Gel Filtration of AS-B in Human Lung Extract on Sephadex G-75

Column size, 15×900 mm. Flow rate, 5 ml/h. Fraction size: 3.0 ml/tube. Eluent: 0.01 M Tris-HCl buffer, pH 8.0, in 0.1 M NaCl.
------, O.D. (280 nm); ●—●, arylsulfatase B activity (1 U = 1 nmol 4-MU·$h^{-1}$·$ml^{-1}$).

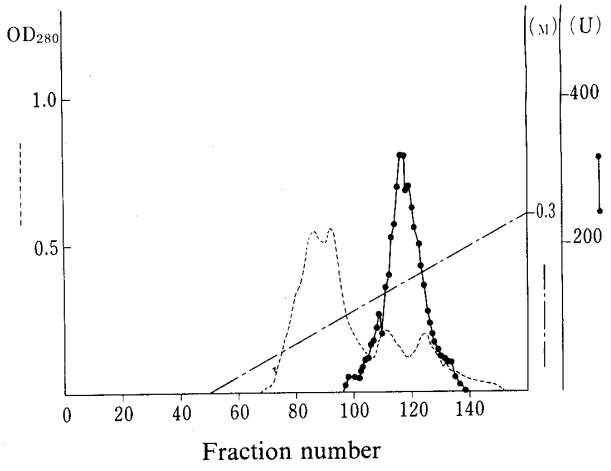


Fig. 2. CM-Cellulose Chromatography of AS-B in Human Lung Extract

Column size: 10×300 mm. Flow rate: 15 ml/h. Fraction size: 3.0 ml/tube. Eluent: 0.05 M acetate buffer, pH 5.0. NaCl gradient: from 0 to 0.35 M.
------, O.D. (280 nm); ●—●, arylsulfatase B activity (1 U = 1 nmol 4-MU·h$^{-1}$·ml$^{-1}$); – – – –, NaCl mol gradient.

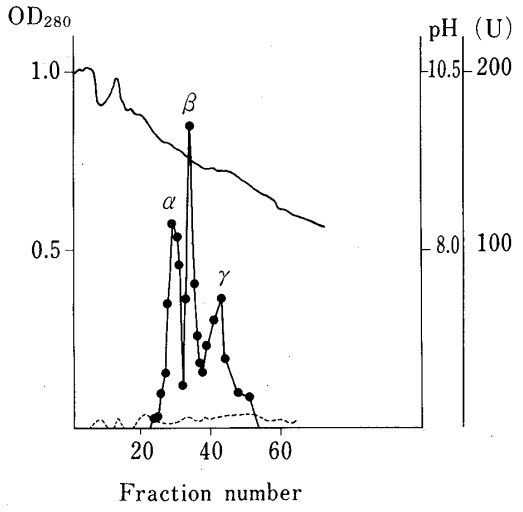


Fig. 3. Chromatofocusing of AS-B in Human Lung Extract

Column size: 10×100 mm. Flow rate: 60 ml/h. Fraction size: 1.5 ml/tube. Eluent: 0.0125 M Pharmalyte HCl buffer, pH 8.5.
------, O.D. (280 nm); ●—●, arylsulfatase B activity (1 U = 1 nmol 4MU·h$^{-1}$·ml$^{-1}$); ——, pH gradient.

to pH 5.5 to 8.0. AS-B from the CM-52 fraction which had been frozen and thawed still gave 3 bands on chromatofocusing, but only one, which was eluted at pH 9.46, was still active.

AS-B has been isolated and purified from the liver,[7] brain[8] and leukocytes,[9] but multibands were still found on ion-exchange chromatography or isoelectric focusing, *i.e.*, 2 bands in the liver, 3 bands in the brain and 2 bands in leukocytes. The pH optima and enzyme kinetics of the AS-B fractions eluted at pH 9.42 and pH 9.06 in the present work were essentially identical and only the isoelectric points were different. Heat stability of the AS-B fraction was examined with the Sephadex G-75 fraction and the maximum AS-B activity was found at 45—46 °C.

In the present study, the chromatofocusing technique was applied to the isolation and purification of AS-B, and rapid separation of AS-B was efficiently and reproducibly performed in 1—2 h. Since the CF column can be readily regenerated *in situ*, the present method may be clinically useful for diagnostic purposes.

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