Arylsulfatase from *Pseudomonas* sp. Strain C₁₂B: Purification to Homogeneity, Immunological Analysis, and Physical Properties

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Arylsulfatase was purified 219-fold from *Pseudomonas* sp. strain C₁₂B. The final preparation was homogeneous by electrophoretic and immunological analysis. The enzyme is a monomer of molecular weight about 51,000, with a Stokes radius of 3.0 × 10⁻⁷ cm, a fractional ratio of 1.2, and a sedimentation coefficient of 4.1S.

Arylsulfatase (arylsulfate sulfohydrolase, EC 3.1.6.1) is a cell wall-associated enzyme in bacteria (5, 10) and catalyzes the hydrolysis of phenolic sulfate esters. Although genetic and physiological factors regulating the synthesis of this sulfatase are known (1, 7, 9–12, 15), the precise physiological function of the enzyme is still undefined (4). Observations that structurally and mechanistically unrelated substances such as tyramine stimulated in vitro activity of partially purified preparations of this enzyme (3, 6) make it essential that homogeneous preparations of arylsulfatase be made available. This need is further emphasized because one form of the sulfatase in *Aspergillus oryzae* may have a sulfotransferase function (2).

*Pseudomonas* sp. strain C₁₂B was derepressed for arylsulfatase synthesis by growth for 16 h at 30°C (in a 400-liter fermentor) on 1 mM methionine as the sulfur source in minimal media containing 1% (wt/vol) sodium citrate (6).

**Enzyme purification.** All procedures were carried out at 4°C.

**Step 1: cell extract.** A thick slurry in 0.01 M Tris-hydrochloride buffer (pH 7.5) was made of the entire cell mass (721 g, wet weight), and this was passed three times through a Manton-Gaulin homogenizer. Cell debris was removed by centrifugation at 39,000 × g for 30 min. Ammonium sulfate was added to the supernatant (1,560 ml) until 90% saturation was attained. After stirring for 4 h, the precipitate was dissolved in 1 liter of 1.6 mM sodium phosphate buffer (pH 7.2), and the solution was dialyzed for 7 days against changes of the same buffer totaling 100 liters.

**Step 2: streptomycin sulfate.** Nucleic acid and protein were precipitated from the extract after dilution with the above buffer to a protein content of 10 mg ml⁻¹ (13). Streptomycin was added to a final concentration of 1 mg mg⁻¹ of protein. After dialysis for 3 days against 0.05 M Tris-hydrochloride (pH 7.5), the ratio of optical density at 280 nm to that at 260 nm of the final supernatant was 1.54.

**Step 3: ammonium sulfate fractionation.** Ammonium sulfate was added to the above supernatant (2 liters) to give 50% saturation. The precipitate which formed after stirring for 4 h was discarded, and the clear supernatant was brought to 90% saturation with this salt. The precipitate was dissolved in 550 ml of and dialyzed for 48 h against frequent changes of the step 2 buffer (20 liters total).

**Step 4: DEAE-Sephadex.** Portions (90 ml) of the step 3 dialysate (1,600 mg of protein each) were applied separately to columns (5 by 45 cm) of DEAE-Sephadex. Before chromatography, the dialysate was maintained at 4°C. Samples were eluted at 60 ml h⁻¹ with 4 liters of a linear (0 to 1 M) NaCl gradient and then washed into columns with 3 void volumes of the step 2 buffer. Fractions (12 ml) were collected as soon as each sample was applied, and most (90%) of the enzyme was eluted in fractions 140 through 150. Fractions from separate runs were pooled and dialyzed against the step 2 buffer.

**Step 5: gel filtration.** After volume reduction to 64 ml (by dialysis against dry carboxymethyl cellulose), the step 4 solution was applied in two parts (300 mg of protein each) to separate columns (5 by 45 cm) of Sephadex G-150 eluted (9 ml h⁻¹) with step 2 buffer. Most of the enzyme was detected in tubes 95 through 115 when 5-ml fractions were collected. These fractions were pooled and dialyzed for 72 h against six 4-liter changes of 0.02 M potassium phosphate buffer (pH 7.6).

**Step 6: carboxymethyl cellulose.** The step

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5 preparation (494 ml; 212 mg of protein) was applied to a column (2.5 by 40 cm) of Bio-Rex 70 eluted (20 ml h⁻¹) with the step 5 buffer. The enzyme was detected at the void volume of the column.

Step 7: DEAE-cellulose. The eluate was dialyzed against 0.02 M phosphate buffer (pH 6.8) until the pH of this solution was 6.8. The solution (850 ml; 140 mg of protein) was applied to a column (2.5 by 40 cm) of Whatman DE-52. After washing with buffer (300 ml), a linear (0 to 1 M) gradient of NaCl (2 liters) was applied, and 12-ml fractions were collected at 20 ml h⁻¹. Fractions (31 through 38) were combined and dialyzed for 72 h against six 4-liter changes of the step 2 buffer.

Steps 8 and 9: DEAE-Sephadex, step elution with NaCl. Eluate samples (49 mg of protein) were applied separately to columns (2.5 by 30 cm) and washed in with 500 ml of the step 2 buffer. Each column was then washed successively with six concentrations (500 ml each) of NaCl (0.125 to 0.2 M with increment increases of 0.015 M). After dialysis to remove salt, the 0.155 M eluate (17 mg of protein) was rechromatographed, and the enzyme was detected in the 0.125 M NaCl wash fluid. Results are given in Table 1.

Criteria for enzyme purity. A single protein band was observed in polyacrylamide gels stained with Coomassie brilliant blue R250 after electrophoresis (14) of the step 9 preparation (125 µg of protein; Fig. 1). A single band was also observed after electrophoresis (18) in the presence of sodium dodecyl sulfate (data not shown). Band diffusion decreased with decreases in sample protein. A discrete line of protein was observed to correspond in parallel gels to a similar line when these gels were stained separately for enzyme (14) and protein. After electrophoresis of samples from all steps, a single band was observed in gels stained for the enzyme.

Antisera were prepared by immunizing rabbits separately against the step 4 and step 9 preparations. Three major precipitin lines and several minor lines were observed after immunodiffusion and reaction of homologous antiserum with the step 4 preparation. Only one precipitin line occurred with the step 9 preparation, and this formed a line of identity with the major lines. After immunoelectrophoresis, a single precipitin line also formed between the step 9 preparation and antiserum against the step 4 preparation (data not shown). When antiserum against the

![Fig. 1. Analysis of samples from various steps in the purification procedure by polyacrylamide gel electrophoresis. Zymograms 1 through 6 correspond to steps 4 through 9 listed in Table 1. Gels were stained for protein by using Coomassie brilliant blue R250.](http://jb.asm.org/)

**Table 1. Purification of arylsulfatase from Pseudomonas sp. C3B**

<table>
<thead>
<tr>
<th>Step and fraction</th>
<th>Total enzyme (units)*</th>
<th>Total protein (mg)</th>
<th>Enzyme recovery (%)</th>
<th>Sp act (units/mg of protein)</th>
<th>Fold purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cell extract</td>
<td>1,269</td>
<td>38,540</td>
<td>100</td>
<td>0.03</td>
<td>1.7</td>
</tr>
<tr>
<td>2. Streptomycin sulfate</td>
<td>1,074</td>
<td>22,000</td>
<td>84.6</td>
<td>0.05</td>
<td>1.7</td>
</tr>
<tr>
<td>3. Ammonium sulfate</td>
<td>1,073</td>
<td>9,477</td>
<td>84.6</td>
<td>0.01</td>
<td>1.7</td>
</tr>
<tr>
<td>4. DEAE-Sephadex</td>
<td>1,051</td>
<td>583</td>
<td>82.8</td>
<td>1.77</td>
<td>59</td>
</tr>
<tr>
<td>5. Gel filtration</td>
<td>373</td>
<td>212</td>
<td>29.4</td>
<td>1.76*</td>
<td>59</td>
</tr>
<tr>
<td>6. Carboxymethyl cellulose</td>
<td>336</td>
<td>140</td>
<td>26.4</td>
<td>2.40</td>
<td>80</td>
</tr>
<tr>
<td>7. DEAE-cellulose</td>
<td>266</td>
<td>99</td>
<td>20.9</td>
<td>2.69</td>
<td>90</td>
</tr>
<tr>
<td>8. DEAE-Sephadex, step elution</td>
<td>187</td>
<td>34</td>
<td>14.7</td>
<td>5.53</td>
<td>184</td>
</tr>
<tr>
<td>9. DEAE-Sephadex, step elution</td>
<td>97</td>
<td>15</td>
<td>7.7</td>
<td>6.57</td>
<td>219</td>
</tr>
</tbody>
</table>

* After each step, sulfatase activity and protein content (6) were determined on samples dialyzed against 0.05 M Tris-hydrochloride buffer (pH 7.8). One enzyme unit is the amount of arylsulfatase required to release 1 µmol of p-nitrophenol from p-nitrophenoxy sulfite in 1 min at 30°C and pH 7.8.

* This fraction had a higher specific activity (2.58) but lost activity during storage.
step 9 preparation was permitted to diffuse to and react with the step 4 preparation, only one precipitin line was observed (Fig. 2).

Physical properties. After sucrose density gradient centrifugation of the step 9 preparation (8), a sedimentation coefficient for arylsulfatase of 4.1S was calculated by using the Svedberg equation. This value, together with the diffusion coefficient for this protein, was utilized to calculate molecular weight (17). Assuming a partial specific volume of 0.725 cm³ g⁻¹, a weight of 52,000 was calculated for this protein. A diffusion coefficient of 7.3 × 10⁻⁷ was calculated (17) from its Stokes radius. This latter value was obtained (17) by determining the average elution position ($K_{av}$) of arylsulfatase from a Sephadex G-200 column previously calibrated by applying and eluting standard proteins of known Stokes radii. The $K_{av}^{1/3}$ of arylsulfatase corresponded to a Stokes radius of 3.0 × 10⁻⁷ cm (Fig. 3).

A fractional ratio for arylsulfatase of 1.2 was calculated (16) from its Stokes radius and molecular weight using the partial specific volume stated above. Since a ratio of 1.0 is given by a perfect sphere (16), the ratio for arylsulfatase suggests that the protein may be nearly spherical in shape. To confirm the value for molecular weight upon which this ratio is based, direct determinations were made by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (18). Determinations for two separate runs gave a weight of 50,000 ± 2,500. The line of best fit (determined by the least-squares method) for the standard proteins utilized gave $r$ and $r^2$ values of 0.996 and 0.992, respectively.

LITERATURE CITED


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