that the same active site was utilized for catalysis of both reactions[1]. Implicit in this hypothesis is the notion that NAD^+ and NADH are bound by the same protein functional groups so that the orientations of the pyridine and dihydropyridine rings are the same with respect to the configuration of the active site. If this hypothesis be true, then hydride ion transfer from glyoxylate to NAD^+ in glyoxylate oxidation should proceed to the same side (A) of the 4 position of the pyridine ring as the side from which a hydride ion is given when glyoxylate is reduced with NADH. This result was found in the oxidation of [^{3}H]glyoxylate with NAD^+ by pig heart lactate dehydrogenase.

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The isolation of arylsulphatase isoenzymes from *Pseudomonas aeruginosa*

The precise nature and specific metabolic function of bacterial arylsulphatase (arylsulphate sulphohydrolase, EC 3.1.6.1) is presently unresolved. Only one such enzyme has been obtained in homogeneous form and many of its properties were found to be distinct from those of mammalian arylsulphatase[1,2]. Other workers have advanced the possibility of multiple forms of this enzyme in *Proteus rettgeri*[3,4] and *Proteus vulgaris*[5] and it has been suggested from studies with cell extracts of *Pseudomonas aeruginosa* that several sulphatases were synthesized depending upon the nature of the sulphur source in the growth medium[6].

The implication of the latter study is confirmed by the present work which describes the first isolation of two highly purified arylsulphatase isoenzymes from a bacterium.

TABLE I

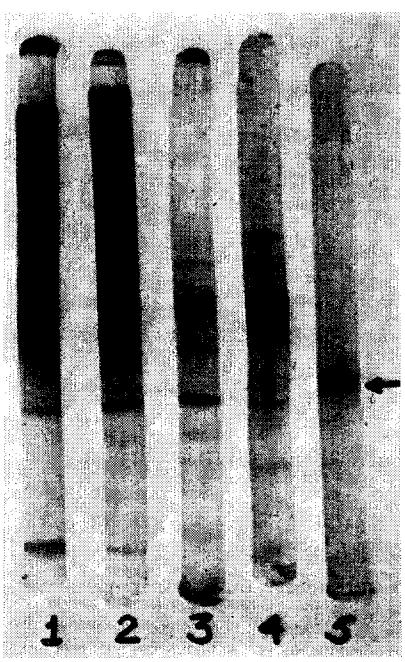
PURIFICATION OF ARYLSULPHATASE FROM *P. aeruginosa*

Fraction	*Total protein (mg)*	*Specific activity (units/mg)*		*Recovery (%)*	
		p-Nitrophenol sulphate	*Nitrocatechol sulphate*	*p-Nitrophenol sulphate*	*Nitrocatechol sulphate*
Acetone powder extract	1539	0.0224	0.0451	100	100
DEAE-cellulose (pH 7.5)	432.8	0.0941	0.1249	118*	78
DEAE-Sephadex (pH 7.5)	199.2	0.137	0.239	79	68
35–70% satd. $(NH_4)_2SO_4$	112.0	0.1862	0.3686	60	59
DEAE-Sephadex (pH 8.8)	26.5	0.7592	1.444	58	55
Prep. acrylamide gel	6.6	2.49	4.464	48	42

* Due to presence of endogenous inhibitor in initial extract.

Enzyme concentration was determined by method of DODGSON AND SPENCER[7] employing 10 mM substrates; *p*-nitrophenol sulphate and nitrocatechol sulphate in 0.05 M Tris–HCl buffer (pH 8.8) at 37.5°. A unit is defined as that quantity of enzyme which hydrolyzes 1 μmole of substrate in 1 min.

Pseudomonas aeruginosa was grown for 16 h at 28° in a citrate–ammonium salts



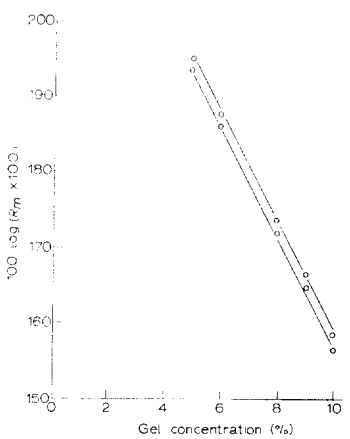


Fig. 1. Disc gel electrophoresis of material from progressive steps in arylsulphatase purification. Samples run in 10% gels with Tris–glycine buffer system of ORNSTEIN AND DAVIS[14] and stained for protein with Amido-Schwartz. Arrow indicates position of enzyme activity in all five gels when duplicate gels stained with *p*-nitrophenol sulphate and nitrocatechol sulphate.

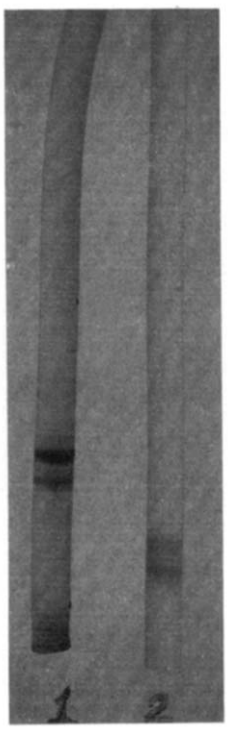
Fig. 2. The effect of different polyacrylamide gel concentrations on electrophoretic mobility of Pseudomonas arylsulphatases[10]. Points represent triplicate determinations at each gel concentration with S.E. $< \pm 0.005$.

medium[8] containing 1 mM $MgSO_4$ and for a further 5 h at 28° in citrate–ammonium salts medium containing 1 mM L-methionine. The enzyme was extracted from an acetone powder of the organism in 0.05 M Tris–HCl buffer (pH 7.5) and purified by DEAE-cellulose, DEAE-Sephadex chromatography using 0.01 M Tris–HCl buffer (pH 7.5) and linear NaCl gradients, 0–1 M and 0.1–0.6 M, respectively. Further purification was achieved by $(NH_4)_2SO_4$ fractionation and preparative gel electrophoresis[9].

There was a 100-fold purification with approximately a 45% yield of enzyme by this procedure (Table I). It should be noted that two proteins which hydrolyzed both substrates were present throughout the purification. The high degree of enzyme purity of the final preparation was reflected by the demonstration of only two protein bands by gel electrophoresis (gel No. 5, Fig. 1).

The same results were obtained with several individual fermentor runs and when different fermentor batches of cells were combined and processed collectively. The finding of two enzymes in a cell extract obtained by sonic oscillation would rule against the possibility that the two enzymes were artifacts arising from the purification procedure.

Since two distinct enzyme bands with slightly different electrophoretic mobili-



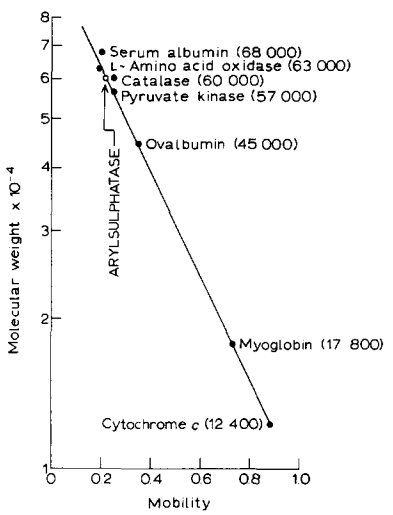


Fig. 3. Acrylamide gel electrofocusing of ovalbumin (300 μg) and Pseudomonas arylsulphatase (50 μg/gel) by method of WRIGLEY[11]. Gels focused at 5° for 16 h at 350 V with anode at top. 1, ovalbumin stained with bromophenol blue; 2, arylsulphatase stained with 0.02 M nitrocatechol sulphate.

Fig. 4. Determination of molecular weight of arylsulphatase by gel electrophoresis using the method of WEBER AND OSBORN[13]. Proteins were run in triplicate gels with S.E. of values obtained $< \pm 0.005$.

ties were noted throughout the purification procedure an attempt was made, using the method of HEDRICK AND SMITH[10], to establish whether they were size or charge isomers or perhaps both. The parallel lines shown in Fig. 2 demonstrate that the two enzymes are charge isomers, they are similar in size but differ in charge. Further investigation of the nature of the charge difference was made by gel electrofocusing[11]. Fig. 3 shows that the charge properties were reflected as a subtle difference in isoelectric point, the p*I* of enzyme I was 4.85 and enzyme II had a p*I* of 4.90.

This degree of resolution in the gel system was also demonstrated with a control protein ovalbumin; the values obtained 4.65, 4.7, 4.58, being identical with those reported by PERLMANN[12].

The molecular weight of the enzyme(s) was found to be approx. 60 000 (Fig. 4) by the acrylamide gel method of WEBER AND OSBORN[13].

The results of this investigation demonstrate that *P. aeruginosa* can synthesize two forms of arylsulphatase (charge isomers) when cultured in a synthetic medium with methionine as the sulphur source. The data would suggest that pseudomonas probably synthesizes a variety of sulphatases with different substrate specificities rather than a non-specific sulphatase.

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