Short Communications

Separation and Partial Characterization of Two Types of Alkaline Phosphatase from *Serratia marcescens*

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The purification and separation of two electrophoretically distinct and chemically different alkaline phosphatases from *Serratia marcescens* is described.

Two types of alkaline phosphatases (EC 3.1.3.1) have been reported in some strains of *Neurospora crassa* (Tohe & Ishikawa, 1971) and *Aspergillus nidulans* (Dorn, 1967). I have found that a certain mutant strain of *Serratia marcescens* synthesizes two electrophoretically distinct phosphatases, F and W. Synthesis of one of them (phosphatase W) was repressed by high phosphate concentration in the growth medium (Bhatti, 1973). The present communication describes a simple and clean separation of phosphatases F and W into chromatographically distinct forms. Some of their distinctive characteristics are also reported.

Strain 211 of *S. marcescens* was a stock culture from the collection maintained by Professor J. L. Still, Department of Biochemistry, University of Sydney. The DE 32 DEAE-cellulose (Whatman) was precycled according to the procedure recommended by the supplier. The column was packed after equilibration with the starting buffer solution (Malamy & Horecker, 1964). The enzyme sample or bacterial suspension (0.5 ml) was assayed for activity in a cuvette of 1 cm light-path by adding 2.5 ml of 1.2 mM *p*-nitrophenyl phosphate in Tris–Mg buffer, pH 8.0 (Tris 10 mM, Mg^{2+} 5 mM, pH adjusted to 8.0, with 1 M-HCl), at 22°C. The change in extinction at 410 nm caused by the release of nitrophenol was measured in a Zeiss spectrophotometer (PMQ II) at 15 s intervals for 3 min. One unit of enzyme activity is defined as that amount of enzyme which liberates 1 μ mol of nitrophenol/min under the prescribed conditions. The specific activity is expressed as units of enzyme activity per mg of protein. Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin (Sigma) as standard. Starch-gel electrophoresis and activity staining were carried out as described previously (Bhatti, 1973). Antiserum to phosphatase F was prepared by the method of Aitken *et al.* (1973).

Strain 211 was cultivated in 28 litres of low-

phosphate medium (Garen & Levinthal, 1960) by continuous-culture procedure (Sayer, 1968). Enzyme was extracted from the cells by a butanol extraction procedure (Bhatti, 1973). The butanol extract was dialysed overnight in Tris–Mg buffer, pH 8.0. Enzyme was precipitated by various concentrations of $(NH_4)_2SO_4$ at 0°C calculated by using the nomogram of Dixon (1953), and further purified by chromatography on columns of DE 32 DEAE-cellulose, by using a linear gradient of NaCl (0–0.2 M) in Tris–Mg buffer, pH 8.0. Fractions (4 ml) were collected with an automatic fraction collector. Effluent flow rate was 4 ml/5 min.

Butanol extraction released 80% of the total enzyme activity present in the cells. There was no sharp precipitation of enzyme with $(NH_4)_2SO_4$. However, 94% of the enzyme activity present in the butanol extract was precipitated between 50 and 80% $(NH_4)_2SO_4$ saturation fractions. Fig. 1 shows the elution profile of the enzyme activity and u.v. absorption at 278 nm in chromatography on DEAE-cellulose. Enzyme activity of phosphatases F and W was separated into two distinct peaks. Starch-gel electrophoresis of different fractions further revealed the separation of two phosphatases. A typical purification is shown in Table 1. Phosphatases F and W are present to the extent of about 1% of the total cellular protein.

Phosphatase F gave maximum activity at pH 9.5, whereas for phosphatase W a broad range of maximum activity was found between pH 8.5 and 10.5. The pH-optimum studies were carried out as described by Wilson *et al.* (1964), but at 22°C and with 1.2 mM substrate concentration. Reversible inactivation at low pH (Schlesinger & Levinthal, 1963) occurred with phosphatase W but very little with phosphatase F. Lowering of the pH to 2.3 possibly caused a conformational change of phosphatase F to a structure that is no longer fully active catalytically (Schlesinger & Barret, 1965; Reynolds & Schlesinger, 1967; Butterworth, 1968). It could also be postulated that subunits formed after acid treatment are sensitive to rapid denaturation and do not recombine

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Table 1. Purification and separation of phosphatases F and W

Purification step	Volume (ml)	$10^{-3} \times$ Total activity (units)	Total protein (mg)	Specific activity (units/mg of protein)
Cell suspension	28 000	142.8	5880	24
Butanol extract	250	114.5	315	363
50–80%-satd.- (NH ₄) ₂ SO ₄ fraction	37	107.8	222	486
DE 32 DEAE-cellulose chromatography				
Phosphatase F	50	43.9	22	1998
Phosphatase W	65	7.9	33.8	235

Fig. 1. Separation of phosphatases F and W by DEAE-cellulose column chromatography

A portion (37 ml) of dialysed 50–80%-satd.- $(\text{NH}_4)_2\text{SO}_4$ fraction was passed through a DE 32 DEAE-cellulose column (2.3 cm $\times$ 17 cm). The column was then washed with 20 ml of Tris–Mg buffer, pH 8.0, as described in the text. $\bullet$, E_{278} ; $\circ$, enzyme activity (units/ml); $\blacktriangle$, NaCl concentration (M). W and F denote the positions of phosphatases W and F.

under experimental conditions (Butterworth, 1968; Glenn & Mandelstam, 1971). Phosphatase F is more thermolabile than phosphatase W. Phosphatase F lost 100% and W only 12% of their enzymic activities after 15 min of incubation at 95°C. The apparent K_m values for *p*-nitrophenyl phosphate were 5.7×10^{-5} M and 8.2×10^{-6} M for phosphatases F and W respectively. Phosphatases F and W were competitively inhibited by orthophosphate, the apparent K_i values being 7.6×10^{-4} M and 5.3×10^{-6} M respectively. Further, antiserum to phosphatase F did not cross-react with phosphatase W, suggesting two

immunochemically different enzyme species. Chromatographically purified phosphatase F from different preparations gave single identical precipitin lines, joining at one point. This further illustrated the homogeneity of these preparations. Reaction of antibody F with butanol extract from different preparations has revealed that about 80–90% of the enzyme activity present in the butanol extract was due to phosphatase F.

The results reported in this communication provide clear evidence for the separation of two electrophoretically distinct enzyme species which are also different in their chemical properties.

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