ACID PHOSPHATASE IN TARAXACUM ROOTS

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Abstract

The enzyme acid phosphatase was studied in Taraxacum *roots with respect to its substrate and pH specificity. A pH optima of ca.* 4.8 *was noted after using ten different phosphate compounds as its substrate. It was found that this enzyme is actively hydrolyzing not only the sugar phosphates but also the nucleotides and coenzymes, releasing free inorganic phosphates, though the rate of the reaction differs with different substrates.*

Introduction

Acid phosphatases which are active in hydrolyzing a variety of phosphate esters in biological systems have been studied in root tissue in the past, with particular reference to their activity in hydrolyzing sugar phosphate esters, (Dyar 1950, Robinson and Brown 1952, and Jenson 1956). Recently Gezelius (1966) used a number of phosphate compounds as substrates for the acid phosphatase of *Dictyostelium discoidum* and found that they were attacked by the enzyme at or near an optimum pH 3.6 but at different rates. It was thought desirable to characterize the acid phosphatase of *Taraxacum* roots with respect to its substrate specificity.

Material and Methods

Whole roots of *Taraxacum officinale* Weber, 7-10 mm dia were collected from a neglected open ground in the vicinity of Sheffield University (U.K.) and brought to the laboratory in polythene bags. After washing the roots thoroughly with tap water, the roots were immediately used for the extraction and estimation of the enzyme acid phosphatase.

Enzyme extraction

The roots were crushed in ice-chilled mortar together with a sufficient quantity (generally 5 ml) of cold (5 C) 0.002 M Tris-HCl buffer, pH 7.4 using acid washed sand. The slurry so obtained was filtered through eight layers of muslin and the filtrate centrifuged at 4000 g for 20 min at 5 C. The supernatant solution was decanted and kept surrounded by ice chips and used for the estimation of the enzyme.

Enzyme assay

Acid phosphatase was estimated by measuring the amount of inorganic phosphate released by the enzyme from the substrate by employing the method of Mitchelson (1957). The reagent mixture was prepared by mixing one volume of 2.34% (W/V) ammonium metavanadate solution with two volumes of 2.5 N HCl. To this, two volumes of 3.53% (W/V) ammonium molybdate solution were added and the mixture diluted to 125 ml with distilled water.

The standard curve of inorganic phosphate was prepared by mixing 5 ml of the above reagent mixture with an appropriate amount of dilute potassium phosphate solution and diluted to 10 ml with distilled water. After standing for 10 min, optical density (O.D.) was measured at 315 mμ against a reagent blank, using 1 cm wide silica cells (Fig. 1).

A 0.5 ml aliquots from the crude enzyme extract was transferred to the graduated test tubes containing 1 ml of 0.2 M citrate buffer with an appropriate pH and 1 ml of 0.01 M substrate solution (phosphate compounds). Following incubation at 37 C for exactly 30 min, the reaction was stopped by adding 1 ml of 20% cold (5 C) trichloro-acetic acid solution. Five ml of the reagent mixture was then added and diluted to 10 ml with distilled water and the O.D. at 315 mμ was measured as for standard curve.

Protein estimation

Total proteins of enzyme extract were determined with the Folin phenol reagent (Lowry *et al.* 1951). The specific activity of the enzyme was expressed as μ M phosphate released per min per mg protein.

Results and Discussion

Using paranitrophenol phosphate (PNP) as reference control, the rate of hydrolysis of other phosphate substrates, namely, Riboflavin-5-monophosphate (R-5-P), Adenosine-2(3')-monophosphate (Yeast adenylic acid), Adenosire triphosphate (ATP), Glucose-1-phosphate (G-1-P), Fructose-6-phosphate (F-6-P), Fructose-1-6-diphosphate (F-1-6-P), Sodium β glycerophosphate (GP), Nicctin amide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP) were estimated at a range of pH's by analysing the phosphate released, using 0.1 M substrate concentrations.

It is clear from the results presented in Fig. 1 that every individual substrate tested was attacked by the enzyme but at varying rates, with an optimum activity at or near pH 4.8 identical to that of PNP. Gezelius and Wright (1965) and Gezelius (1966) demonstrated acid phosphatase in the differentiating cellular slime mold *Dictyostelium discoidum* with a pH optima at ca. 3.6, using PNP

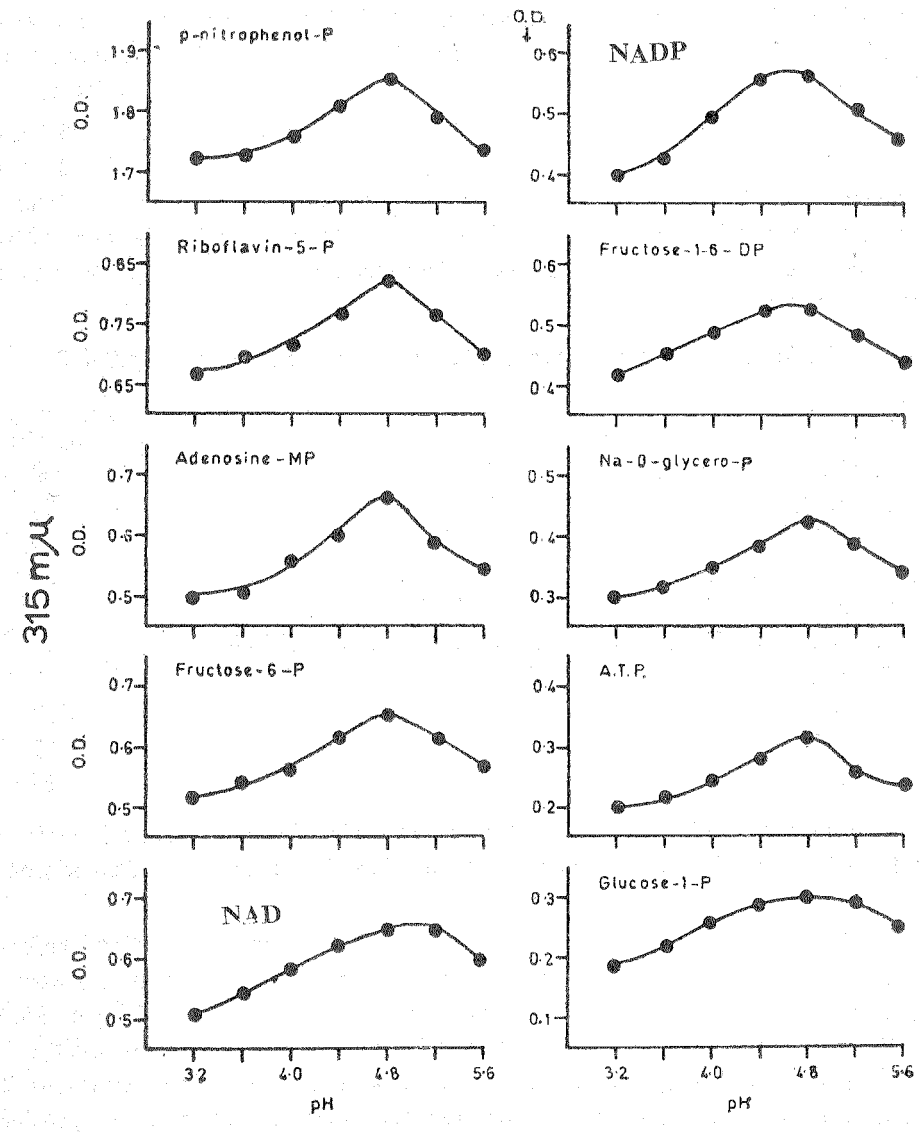

Fig 1 Action of acid phosphatase on a number of substrates at a range of pH's

G-6-P, F-1-6-P and GP as substrates. However, in the present investigation the pH optima of acid phosphatase extracted from *Taraxacum* roots was found around pH 4.8 after using ten different substrates. This difference in pH optima is probably due to specific metabolism of different organisms.

The results of relative substrate specificity of acid phosphatase at PH 4.8 are presented in Table 1. It was found that among sugar phosphates, F-6-P and F-1-6-P are hydrolysed more actively by the acid phosphatase of *Taraxacum* roots as compared to G-1-P. In this respect it resembles with the acid phosphatase of *Dictyostelium discoidum* (Gezelius 1966) except that the percentage of the enzyme was slightly lower.

Table 1. *Relative substrate specificity of acid phosphatase at pH* 4.8

No.	*Substrate*	*Percentage activity*
1.	Paranitrophenol phosphate (PNP)	100
2.	Riboflavin-5-monophosphate (R-5-P)	44
3.	Adenosine -2(3')-monophosphate (Yeast adenylic acid)..	36
4.	Fructose-6-phosphate (F-6-P)	35
5.	Nicotinamide adenine dinucleotide (NAD)	35
6.	Nicotinamide adenine dinucleotide phosphate (NADP)	30
7.	Fructose-1-6-diphosphate (F-1-6-P)	29
8.	Sodium β glycerophosphate (GP)	23
9.	Adenosine triphosphate (ATP)	17
10.	Glucose-1-phosphate (G-1-P)	16

The present study has demonstrated that the acid phosphatase is actively hydrolysing not only the sugar phosphates but also the nucleotides and coenzymes, releasing free inorganic phosphates.

Acid phosphatases have a digestive task of the cell contents, and are localized at the cell surface of yeast (Weimberg and Orton 1964), epidermis, meristems, outer and inner cortical tissues of *Vicia faba* root tips (Gahan and Maple 1966). Preliminary observation dealing with the localization of acid phosphatase in *Taraxacum* root cells by the method of Issac and Winch (1947) indicated its association with the cell wall of phloem parenchyma suggesting its possible digestive role.

The present study shows that depending upon the place of their localization and the kind of substrate available in the plant tissue a slight change in pH opitma could be speculated.

Acknowledgments

The author wishes to express his gratitude to Dr. A. Booth for supervising this work and also to his elder brother Dr. Mohammad Idrees Khan for the financial support during this investigation.

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