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A new repressible alkaline phosphatase in *Neurospora crassa* EM 5297a

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Abstract. *Neurospora crassa* Em 5297a can utilize sodium β-glycerophosphate as a sole phosphorous source (in the place of KH_2PO_4). Under these conditions a repressible alkaline phosphatase is elaborated which has different pH optimum towards β-glycerophosphate (10.2) and pyrophosphate (9.0) as substrates. This enzyme does not require any metal ion for its activity and could be assayed in the presence of EDTA. However, under conditions of cobalt toxicity, the activity of this enzyme is high and is decreased in copper and nickel toxicities.

Keywords. *Neurospora crassa* Em 5297a; repressible alkaline phosphatase; β-glycerophosphate.

Introduction

Recent studies have revealed the presence of inorganic phosphate repressible alkaline and acid phosphatases in *Neurospora crassa* (Nyc *et al.,* 1966; Kader *et al.,* 1968; Nyc, 1967; Jacobs *et al.,* 1971). Unlike the constitutive enzyme, the repressible alkaline phosphatase of *N. crassa* 1A has no metal requirement and is distinguished from the former by the fact that it is assayed in the presence of EDTA (Nyc *et al.,* 1966). In contrast to the above, we found in *N. crassa* Em 5297a a repressible alkaline phosphatase elaborated when this strain was grown on β-glycerophosphate as a phosphorous source. This phosphatase differs in certain of its properties from the constitutive alkaline phosphatase of Em 5297a (Ku and Blumenthal, 1961) as well as the repressible enzyme of *N. crassa* 1A (Nyc *et al.,* 1966). Some features of this new enzyme are reported in this paper.

Materials and methods

Chemicals

All chemicals including sodium β-glycerophosphate and pyrophosphate were of Analytical grade and were obtained from British Drug House, India.

Organism and growth conditions

A wild strain, *Neurospora crassa* Em 5297a used in the present studies was maintained by weekly subcultures on agar slants. It was grown for 72 h at 30 ± 1° C in 50 ml conical flasks containing 10 ml basal medium. The medium composition was as described earlier except that it contained 0.04% disodium β-glycero-

phosphate monohydrate and 0.015% KCl in the place of KH_2PO_4 (Venkateswerlu and Sivarama Sastry, 1973). To study the effect of metal toxicities on the elaboration of the repressible phosphatase, Co^{2+}, Ni^{2+} and Cu^{2+} were also included at 600, 300 and 800 μg/10 ml respectively in the culture medium. The pH of the medium was always adjusted to 4.5-5.0.

The mycelia (72 h old) were washed with ice-cold distilled water, pressed dry between the folds of filter paper and ground at 0-4°C with twice their weight of glass powder in a mortar and pestle with 0.5% sodium deoxycholate, pH 7.0 (5ml/ 150 mg fresh mycelial weight). The homogenate was centrifuged at 4°C for 15 min at 10000 *g* and the supernatant collected. The residue was reextracted likewise with another 2-3 ml of deoxycholate. The pooled extracts constituted the enzyme source. The protein content of the extract was determined by the method of Lowry *et al.* (1951).

Enzyme assay

Enzyme activity was assayed according to the procedure described by Nyc *et al.* (1966). The incubation mixture contained 10 μmol of sodium β-glycerophosphate (or sodium pyrophosphate as needed), 1 μmol EDTA, 0.1 ml of 0.2 M glycine-NaOH buffer, pH 10.2 (or pH 9.0 when sodium pyrophosphate was the substrate) and a suitable aliquot of enzyme extract in a total volume of 0.9 ml. Incubation was for 20 min at 37°C and the reaction was terminated by adding 0.1 ml of 50% trichloroacetic acid. The tubes were centrifuged and the liberated inorganic phosphate was determined in the supernatant by the method of Fiske and Subba Rao (1925). The specific activity of the enzyme is expressed as μmol of P_i liberated per mg of protein in 20 min.

The constitutive alkaline phosphatase of *N. crassa* is not assayable in the presence of EDTA and hence this assay differentiates between the constitutive and repressible enzymes in question.

All experiments were repeated a minimum of four times, and values were invariably reproducible to within 5-10%.

Results and discussion

The presence of a repressible acid phosphatase has been reported by Grove and Marzluf (1980) in *N. crassa* cultured in the presence of ribonucleic acid as phosphate and nitrogen sources. We report repressible alkaline phosphatase in *N. crassa* Em 5297a, during growth on sodium β-glycerophosphate as a sole phosphorous source.

This enzyme was not detectable in mycelial extracts prepared from *N. crassa* cultures grown in presence of inorganic phosphate in the medium. This indicates that inorganic phosphate represses the phosphatase reported here and that it is similar to the enzyme studied by Nyc *et al.* (1966) in this respect.

The growth response of *N. crassa* Em 5297a towards β-glycerophosphate as a sole phosphorous source and the enzyme activity under these conditions are shown in figure 1. It is interesting that whereas growth is a function of

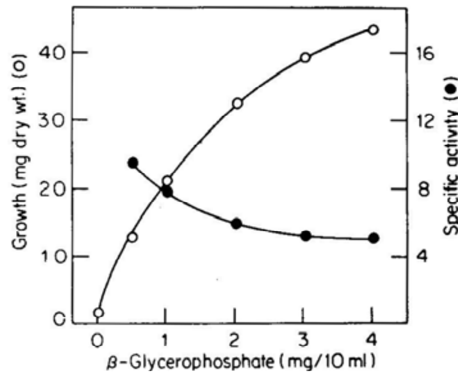

Figure 1. The growth of *N. crassa* on β-glycerophosphate and the activity of a repressible alkaline phosphatase. The mold was grown on 10 ml basal medium containing varying amounts of sodium β-glycerophosphate and 0.015% KCl for 72 h in two sets of flasks. One set of mycelia was used for determining dry weights (O) and the other for enzyme assay (●) as described in text.

glycerophosphate concentration in the range 0–4mg/10 ml medium, activity of the repressible phosphatase is highest with low concentrations of glycerophosphate under conditions of suboptimal growth. Since the enzyme is absent during growth on KH_2PO_4 and is elaborated only on glycerophosphate as the phosphorous source, the data suggest that intracellular inorganic phosphate formed probably represses the enzyme *in vivo*.

The pH optimum for this enzyme was determined using sodium β-glycerophosphate and sodium pyrophosphate as substrates. The results are shown in figure 2.

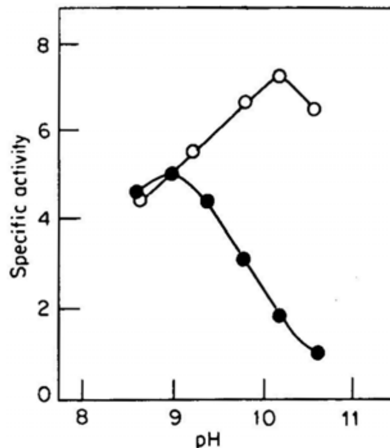

Figure 2. pH optima of repressible alkaline phosphatase from *N. crassa* Em 5297a. The enzyme preparation was obtained from *N. crassa* mycelia, grown for 72 h on 10 ml of basal medium containing 0.04 % β-glycerophosphate. The enzyme assay was as described in text using either β-glycerophosphate (O) or pyrophosphate(●) as substrates.

The phosphatase from strain Em 5297a exhibits pH optima of 10.2 and 9.0 for glycerophosphate and pyrophosphate respectively, whereas the enzyme reported by Nyc *et al.* (1966) exhibited only one pH optimum for both the substrates. Further, this enzyme is 70% as active towards sodium pyrophosphate as towards sodium β-glycerophosphate (enzyme activities obtained at the respective pH optima have been compared.) In contrast, the enzyme from strain 1A has been reported to be only 28 % as active towards pyrophosphate (Nyc *et al.* 1966). The K_m Value for the enzyme from strain Em 5297a is 3.68 mM with β-glycerophosphate as the substrate. The differences between the repressible alkaline phosphatases of *N. crassa* Em 5297a and strain 1A are summarized in table 1 and indicate that the two enzymes are somewhat different in their properties.

Table 1. Comparison of Properties of the repressible alkaline Phosphatases of *N. crassa* strains Em 5297 a and 1A.

	Property	Em 5297a	1A*
1.	pH Optimum with		
	a) β-Glycerophosphate	10.2	9.0
	b) Pyrophosphate	9.0	9.0
2.	% Activity towards		
	a) β-Glycerophosphate	100.0	100.0
	b) Pyrophosphate	70.0	28.0
3.	Km value (For β-glycerophosphate)	3.68 mM	1.0 mM

* Values taken from reference 1.

N. crassa Em 5297a was grown in presence of 0.04% β-glycerophosphate for 72 h and the enzyme was assayed using β-glycerophosphate or sodium pyrophosphate as described in text.

Although, the repressible alkaline phosphatase of *N. crassa* has no metal requirement for activity, Hochberg and Sargent (1973) reported that iron and zinc enhance the synthesis of this enzyme and that copper inhibits its synthesis. In parallel studies to examine the effect of some metal ions we found that enzyme activity in copper and nickel toxic conditions in *N. crassa* Em 5297a was only 15% and 87% of control cultures (see table 2). However, a 50% increase in the enzyme activity was observed in the case of cobalt toxic cultures. These observations indicate that some metal ions like iron, zinc and cobalt may have some role in the biosynthesis of this enzyme as suggested by Hochberg and Sargent (1973).

Table 2. Effect of metal toxicities on the new repressible alkaline phosphatase from *N. crassa* Em 5297a.

Metal	Conc. of metal ion during growth (μg/10 ml medium)	Specific activity
None	Control	7.15 (100)
CO^{2+}	600	10.20 (142.7)
Ni^{2+}	300	6.22 (87.0)
Cu^{2+}	800	1.05 (14.80)

The organism was grown on 10 ml basal medium containing the concentration of metal ions as indicated above, which resulted in 50% growth inhibition along with 0.04% β-glycerophosphate for 72 h. The enzyme was assayed as described in the text. Numbers in parentheses are values expressed as per cent of control.

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