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XANTHINE OXIDASE IN LENTIL (*LENS ESCULENTA*) SEEDLINGS

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Summary

Until recently there were no reports regarding the presence of xanthine oxidase (xanthine:oxygen oxidoreductase, EC 1.2.3.2) in plants. Direct evidence for its presence in lentil seedlings is reported here. Xanthine oxidase activity increases with the period of germination, reaching a maximum at 24 h and decreasing thereafter. The pH optimum for its activity is at pH 8.0. Almost equal activity is observed against xanthine and hypoxanthine. The K_m for xanthine is 1.05 mM, and considerable inhibition is observed at high substrate concentration.

Xanthine oxidase (xanthine:oxygen oxidoreductase, EC 1.2.3.2) and the closely related enzyme, xanthine dehydrogenase have been known to occur for a long time in animals and bacteria [1]. Until recently, there were no reports regarding their presence in higher plants. Xanthine oxidase was suggested to be present in pea seedlings infiltrated with hypoxanthine, on the basis of the detection of xanthine; but uric acid could not be detected as a metabolic product [2]. Recent experiments with ^{14}C-labelled xanthine and hypoxanthine indicate that their metabolism in excised tips of tea shoots may be similar to that in animals [3]. We now report direct evidence for the presence of xanthine oxidase, catalysing the aerobic oxidation of hypoxanthine and xanthine to uric acid, in lentil seedlings.

Lentil seeds were surface sterilised as described by Ahlowalia and Mallar [4], soaked in distilled water for 4 h and placed on moist blotting paper. These were harvested after 24 h. Seedlings were washed and a 50% homogenate prepared in 10 mM Tris·HCl buffer, pH 7.5, in a chilled mortar and pestle. The homogenate was filtered through double layered muslin cloth and then centrifuged at 15 000 rev./min for 20 min at 0—2°C. The resulting supernatant was used for enzyme assay.

Enzyme activity was determined spectrophotometrically by measuring the increase in absorbance at 290 nm consequent to aerobic oxidation of

hypoxanthine/xanthine to uric acid, according to the method of Kalckar [5]. Tris·HCl buffer 120 μmol, pH 7.5/8.0, 0.9 μmol xanthine/hypoxanthine and suitably diluted enzyme in a total volume of 2 ml were incubated at 37°C for 15 min. The reaction was stopped by the addition of 1.0 ml 10% perchloric acid. In the control experiments substrate was added after the addition of perchloric acid. After centrifugation at 5000 rev./min for 15 min the supernatant was suitably diluted and absorbance measured at 290 nm in a Beckman DB-G spectrophotometer. Under the conditions of the absorbance measurement after the addition of perchloric acid, namely pH 1.0, the difference between the molar extinction coefficient ($\epsilon_{290\,nm}$) of uric acid and xanthine is $19 \cdot 10^3$ $cm^{-1} \cdot M^{-1}$. The increase in absorbance at 290 nm is proportional to the period of incubation and the enzyme concentration. An enzyme unit is defined as the amount of enzyme required for the formation of 1 μmol of uric acid in 15 min at 37°C. Protein was determined by the method of Lowry et al. [6].

Uric acid formed during the reaction has also been identified by paper chromatography. Suitably diluted enzyme and xanthine (100 μmol) were incubated for 1 h at 37°C in 140 μmol Tris·HCl buffer, pH 7.5, total volume 2 ml. The reaction was stopped by adding 1.0 ml 4.5% barium hydroxide followed by 1.0 ml 5% zinc sulphate. In the control experiment, substrate was added after addition of barium hydroxide and zinc sulphate. After centrifugation, the supernatant was concentrated and applied to Whatman chromatographic paper No. 1. Standard xanthine and uric acid were also applied simultaneously. Ascending chromatography was carried out in water-saturated *n*-butanol/formic acid (1:1, v/v) solvent system (Block et al. [7]). R_F values for xanthine and uric acid were 0.87 and 0.50 respectively.

Xanthine oxidase activity increases with the period of germination reaching a maximum at 24 h and decreasing thereafter. The protein concentration

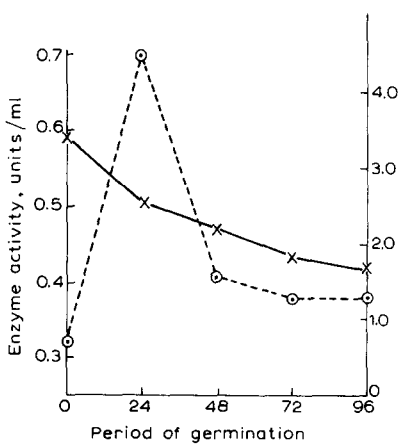

Fig. 1. Effect of period of germination on xanthine oxidase and soluble protein. The activity against xanthine at pH 7.5, determined as described in the text, is expressed in units/ml extract (o- - - -o) and protein as mg/ml extract (x——x).

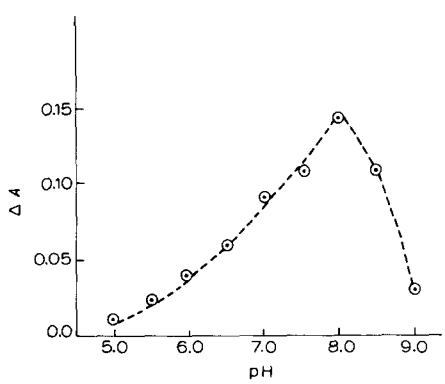

Fig. 2. Effect of pH on xanthine oxidase. The activity against xanthine in sodium acetate/acetic acid buffer (pH 5.0—6.5), phosphate buffer (pH 6.5—7.5) and Tris·HCl buffer (pH 7.5—9.0) was determined as described in text. Protein in 2 ml assay mixture was 0.25 mg. Velocity is expressed in absorbance reading at 290 nm.

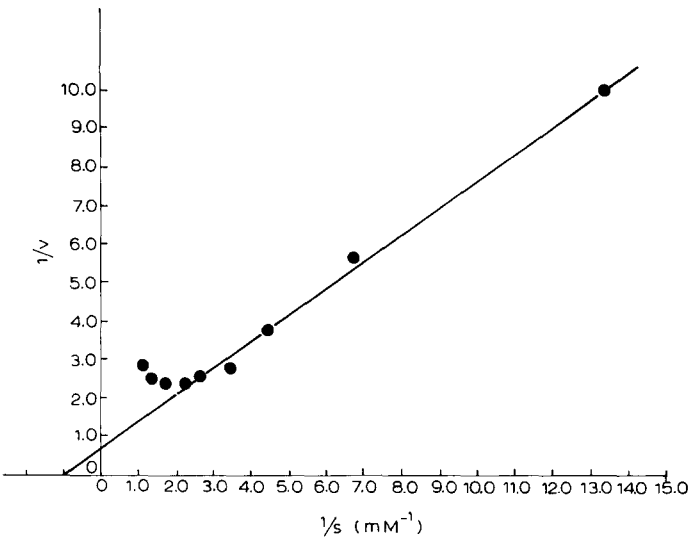

Fig. 3. Lineweaver and Burk plot for xanthine oxidase activity against xanthine at pH 8.0. Protein in 2 ml assay mixture was 0.5 mg. Activity was determined as described in text and expressed in absorbance reading at 290 nm.

on the other hand decreases progressively (Fig. 1). The pH vs. enzyme activity profile (Fig. 2) shows an optimum at pH 8.0. Xanthine oxidase activity against hypoxanthine and xanthine is more or less the same, namely 0.54 and 0.68 unit/ml, respectively. A marked inhibition in enzyme activity is observed at high substrate concentration (Fig. 3) and the K_m value calculated from low substrate concentration data with xanthine at pH 8.0 is 1.05 mM.

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