AN OXALATE OXIDASE FROM GRAIN SORGHUM 
LEAF: USE IN DETERMINATION OF 
URINARY OXALATE

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ABSTRACT

An Oxalate oxidase (Oxalate : O₂ oxidoreductase, EC 1.2.3.4) has been purified to apparent homogeneity from leaves of 10-day old seedling plants of grain sorghum hybrid CSH-5. The enzyme exhibited maximum activity at pH 5.0 and 40°C. The rate of O₂₂ formation was linear up to 2 min. The enzyme was strongly stimulated by Cu++. The enzyme has greater resistance towards various cations and anions found in urine, compared to moss, barley, banana peel and beet stem oxalate oxidases. This improved characteristic of the enzyme make it better suited for its use in the determination of urinary oxalate. A simple method of measuring oxalate in urine using this enzyme preparation is described.

INTRODUCTION

Oxalate oxidase has attracted the attention of several workers since this enzyme from barley seedlings was introduced as analytic reagent for oxalate determination in urine, which is required for the diagnosis and medical management of hyperoxaluria and calcium oxalate urinary calculous disease (1). So far oxalate oxidase from barley seedlings (2), mosses (3), beet stem (4) and banana peel (5) have been employed for urinary oxalate analysis. However these methodologies require the removal of a number of cations and anions normally found in urine, prior to oxalate analysis, which otherwise interfere in the assay. We have purified an oxalate oxidase from leaves of grain sorghum hybrid CSH-5 which is unaffected by a range of inorganic ions normally found in urine, and therefore, more suitable for oxalate determination than other oxalate oxidases. In the present communication we report a simpler method of determination of oxalate in urine employing this enzyme.

MATERIAL AND METHODS

Chemicals : Sphadex G-200, DEAE-Sephadex (Pharmacia, Sweden), 4-aminophenazine, horseradish peroxidase, oxalic acid, L-cysteine, FAD, FMN (Sigma Chemical Co, USA) were used. All other chemicals used were of AR grade. Seeds of grain sorghum (Sorghum vulgare L-hybrid CSH-5) were supplied by Nath Seeds Private Ltd. Aurangabad, India.

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The crude enzyme was prepared in cold from leaves of 10-day old seedling plants of grain sorghum CSH-5 raised in the laboratory as described by Pundir & Nath, 1984 (6).

Purification of Oxalate oxidase: All the steps of purifications were carried out at 4°C. Solid ammonium sulphate was added to crude enzyme to give a final 0-80% saturation. The resulting solution was centrifuged at 10000xg for 30 min. The pellet was dissolved in distilled water, dialysed against 0.01M potassium phosphate buffer (pH 6.7) for 16 hr and loaded onto DEAE-Sephacel column (2.5x29 cm) previously equilibrated with the same buffer. The enzyme was eluted with a linear gradient of NaCl from 0-0.6M in the dialysing buffer. Fractions were collected at a rate of 0.5ml/min. The fractions with high enzyme activity were pooled and applied on a Sephadex G-200 column (1.5x45 cm) equilibrated with 0.02M potassium phosphate buffer (pH 7.2). The active fractions were pooled and treated as purified enzyme. It was stored at -20°C until use.

Enzyme assay: The assay of oxalate oxidase was carried out according to the method of Pundir & Kuchaal, 1989 (7). The enzyme was assayed in 15 ml glass tubes wrapped with black paper. To each tube was added 80 μmol of sodium succinate buffer (pH 5.0), 1.0 μmol of oxalic acid and enzyme protein in total volume of 2.0 ml. One μmol of CuSO₄ was also added. After incubation at 40°C for 2 min, 1.0 ml of color reagent was added to each tube. The tubes were shaken and kept at room temperature in dark for 30 min. A₅₅₀ was read and H₂O₂ generated was extrapolated from a standard curve of H₂O₂ prepared in 50mM sodium succinate buffer (pH 5.0).

Collection of urine samples: 24 hours urine samples were collected from healthy as well as kidney stone patients in plastic bottles containing 15 ml concentrated HCl.

Determination of urinary oxalate: Acidified urine sample was diluted with an equal amount of phosphate buffer (0.1M, pH 7.0) and the pH of the urine sample was adjusted between 5 to 7 by HCl or NaOH. To avoid possible ascorbate interference, 200mg of activated charcoal was added, sealed with parafilm and mixed for 5 min. The sample was filtered through Whatman No.1 and filtrate was used for oxalate analysis (8).

Assay of urinary oxalate: It was done as described in enzyme assay by using 0.1 ml of urine (filtrate) sample in place of oxalate. The oxalate values in urine were determined from standard curve between oxalate oxidase activity vs. oxalate conc. ranging from 0.1 mM to 1.2 mM.

RESULTS AND DISCUSSION

Results of a typical purification procedure is given in Table 1. The overall purification achieved was 113 fold with an activity yield of 8.8%. The purified enzyme appeared to be homogenous since only a single protein band was observed by polyacrylamide gel electrophoresis.

Enzyme properties: The optimum activity of the enzyme was at pH 5.0. The enzyme was stable in pH ranges from 4 to 6. The pH optima in acidic pH ranges has been reported for barley seedlings (9), mosses (3) and Pseudomonas sp. OX-53 (10). The rate of the reaction was linear upto 2 min after which there was a slight decrease in the activity which is five times less than the time required for barley enzyme. The enzyme activity was inhibited by EDTA indicating the metal ion requirement. The strong inhibition by DEDTC, 8-hydroxyquinoline and their partial reversal by Cu²⁺ only revealed by Cu²⁺ requirement of the enzyme. Among the metal ion tested, only Cu²⁺ caused 300% stimulation (Table 2). Other metals such as Na⁺, K⁺, Mg²⁺, Ca²⁺, Zn²⁺, Mn²⁺, Fe²⁺, Pb²⁺ had no effect. Further, the enzyme was unaffected by chloride ions as