Comparative Study of Acid phosphatases from Maize Millet and Sorghum Seeds

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Introduction

Rainfed area crops including maize (Zea mays), millet (Pennisetum americanum) and sorghum (Sorghum vulgare) are the important cereal crops in Pakistan. These are also cultivated in North West Frontier Province of Pakistan where it forms an important part of the diet of the rural population.

In a study on rainfed area crops seeds for determining the suitability of its diet, the seeds were found to contain considerable amount of non-specific acid phosphatase activity. Investigations were, therefore, initiated in order to isolate, purify and characterize the acid phosphatases from maize, millet and sorghum seeds.

Earlier a number of seeds have been reported to contain acid phosphatase activity [1,2] and some of these phosphatases have been studied in detail. Recently a study on acid phosphatase purification and characterization from maize scutellum after 96h germination [3,4] has also been reported. The present study was undertaken to compare the purification scheme and some properties of maize seed acid phosphatase with that of millet and sorghum seeds.

Experimental

The following reagents were purchased from the sources indicated:- p.nitrophenyl phosphate (disodium salt), Bovine Serum albumin were from Merck; DEAE-Cellulose was from Sigma, Sephadex G-200 and Blue dextran from Pharmacia. All other reagents were of guaranteed reagent grade.

Assay Procedures:

Acid phosphatase activity was determined by method of Bessey et al. [5]. Enzyme assays were run in 0.1M acetate buffer (pH 4.9) using 1 ml 1mM p.nitrophenyl phosphate as substrate at 37°C. The reaction was stopped after incubation for 30 minutes by the addition of 5ml 1N NaOH and p.nitrophenol absorbance was measured at 405 nm.

Protein concentration was estimated from absorbance at 280 nm. or by the method of Lowry et al. [6] with slight modification using bovine serum albumin as standard.

The buffers used to cover the pH range required were acetate buffer (pH 4 - 5.8), maleate buffer (pH 5.8 - 7.0) and Tris-HCl buffer (pH 7 - 9). In the study of inhibition experiments, final concentrations of inhibitors were adjusted to 0.5 mM and 1 mM in all cases.

Enzyme purification:

All purification steps were carried out at 4°C, enzyme activity was

determined at each stage of purification. High yielding and approved varieties for whole of N.W.F.P. of maize (Sarhad white), millet (DB-2) and sorghum (Giza) were collected from Agriculture research Institute, D.I.Khan.

Seeds were ground to rough powder followed by extraction with diethyl ether to remove lipid contents. The sample was air dried and then ground to fine powder. Delipidation was carried out only in case of maize. The crude enzyme was extracted by shaking the powder in 0.01 M Tris-HCl buffer pH 7.0 (0.5g powder/10 ml buffer) for 1 h at 4°C. The mixture was then centrifuged at 5000 rpm for 10 minutes. The supernatant, thus obtained was used for partial purification of the enzymes. The supernatant was dialysed against 0.01 M Tris-HCl buffer pH 7.0 (with several changes of buffer). The dialysed protein fraction was again centrifuged at 5000 rpm for 10 minutes to remove the precipitated proteins and the supernatant (25 ml) was applied to DEAE-cellulose column (2x20 cm) previously equilibrated with 0.01 M Tris-HCl buffer pH 7.0. Elution was performed with the same buffer at flow rate of 10ml/10 minutes (10 ml fractions). Following this, a linear salt gradient (0-1 M NaCl) in the same buffer was applied. The tubes representing enzyme peak were pooled, concentrated under vacuum distillation and applied on to a column of Sephadex G-200 (2 x 100 cm) previously equilibrated and eluted with 0.01M Tris-HCl buffer pH 7.0 at flow rate of 10 ml/15 minutes (5 ml fractions). The tubes representing enzyme peak were pooled, dialyzed against distilled water, freeze dried and stored for further study of characteriztion.

Results and Discussion

Acid phosphatases from three sources were purified with uniform and similar procedure to make an appropriate comparison of their properties and characteristics.

Purification was achieved by ion-exchange chromatography on DEAE-cellulose. The enzyme was completely bound while the unwanted proteins were washed out with equilibration buffer. The enzyme was eluted with linear salt gradient and all the enzyme activity was eluted between 0.1M - 0.2M NaCl in the same buffer. 2 - 3 times purification of the enzyme was achieved with 67 - 90% yield in all three cases. The active fractions of anion exchange chromatography were subjected to Gel filtration on Sephadex G-200 column. The enzyme activity came out as one peak with 10 - 15 times purification. The results of partial purification of acid phosphatases from various sources are summarized in table 1.

Purified enzymes from different sources seemed to be more stable at 4°C. No activity loss could be detected even after incubation at 20°C for 24 h. At high temperatures activity decrease was significant in all cases. After incubation period of 1 h at 40°C enzymes lost 20 - 25% of its activity and at 60°C, the activity decrease was 90-95%. At 70°C, the enzymes appeared to be completely denatured after 10 minutes. All enzymes showed a broad region of great stability between pH 4.5 - 8.5. The pH optima were measured over pH values ranging from 3.8 - 6.5. Optimum pH (4.9) was the same for all enzymes studied. When temperature optima were determined, no significant differences were seen in the profiles. Temperature optima were found to be in all cases near to 55°C. Above this temperature, the activity was decreased very sharply which may be due to heat denaturation of the enzymes. K_m values against p.nitrophenyl phosphate were also identical and are shown in table 2. The kinetic study of the inhibitory

Table-1: Purification steps of maize, millet and sorghum acid phosphatases.

Purification steps	Maize		Millet		Sorghum	
	P.F	Act.Rec.	P.F	Act.Rec.	P.F	Act.Rec.
1.DEAE-cellulose	2.1	90%	3.2	67%	3.0	80%
2.Sephadex G-200	30.0	67%	36.0	55%	29.0	64%

P.F :- Purification factor Act.Rec.:- Activity recovery.

Table-2
Comparison of K_m values and K_i values for various inhibitors against p.nitrophenyl phosphate as substrate.

Enzyme	K_m (mM)	K_i for fluoride ion (mM)	K_i for phosphate ion (mM)
Maize	0.25	0.28	0.55
Millet	0.28	0.68	1.00
Sorghum	0.36	0.39	0.42

Each value is the mean value of three determinations.

action of some compounds revealed predominantly competitive or non-competitive types of inhibitors. The inhibition constants (K_i) were calculated from Lineweaver-Burk plots [7] and Dixon plots [8]. Sodium phosphate, a well-known inhibitor of acid phosphatases displayed the same effect on the three enzymes. A competitive type of inhibition was observed. All enzymes also showed identical responses towards sodium fluoride. Inhibition produced by NaF was non-competitive. The inhibition constants for all inhibitors are indicated in table 2.

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