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CHANGE IN THE ENZYMATIC ACTIVITY OF NADP$^+$ DEPENDENT ISOCITRATE DEHYDROGENASE IN GERMINATING SEEDS OF BALCKGRAM

M Ayub Ali*, W joykumar Singh, L Inatombi Devi and L Rupchandra Singh
Department of Biochemistry, Manipur University, Canchipur - 795 004, Manipur

ABSTRACT

The NADP$^+$ -dependent Isocitrate Dehydrogenase was extracted from germinating seeds of black gram [*Vigna mungo* (L) Hepper] at different time periods of germination. The specific activity of extracted enzyme sample was found to be vary as a function of the time period of germination. It increased from 0.02 units/mg protein at 0 hour to 0.52 units/mg protein at 48 hours of germination and then declined at longer periods of germination. At 84 hours of germination, the specific activity stood at 0.15 units/mg protein only.

INTRODUCTION

The NADP$^+$ -dependent Isocitrate Dehydrogenase [*threo*-D_s-Isocitrate: NADP$^+$ oxidoreductase (decarboxylating), Ec 1.11.42 (NADP$^+$-IDH) catalyses the oxidative decaboxylation of isocitrate to yield -ketoglutrate with concomitant reduction of NADP$^+$ to NADPH. The enzyme requires metal ion (Mn^{2+} or Mg^{2+}) for catalysis, presumably to coordinate with enzyme-bound substrate and act as super acid (walsh, 1979). The enzyme reported long ago, is now known to be present in plant cytosol and root nodules (Henson et al, 1986), chloroplasts (Galvez et al, 1994), mitochondria (Rasmusson and Moller, 1990), peroxisomes (Randall and Givan, 1981), germinating and maturing seeds (Satoh, 1980) and roots (Chen et al, 1988). The NADP$^+$ - Isocitrate Dehydrogenase from plant seeds changes its activity during germination. The present paper documents the variation in enzymatic activity of the NADP$^+$ - isocitrate dehydrogenase during germination of blackgram at Biochemistry Laboratory, Manipur University, Canchpur.

MATERIALS AND METHODS

Enzyme assay

The activity of NADP$^+$ -dependent Isocitrate Dehydrogenase was measured spectrophotometrically according to the method of Curry and Ting (1976) by monitoring the rate of formation of NADPH (NADH at 340 nm = 6.22 x 103 M-1 cm-1) in a reaction mixure (0.8 ml) containing appropriately diluted enzyme, 2.0 mM DLisocitric acid (equivlent to 1.0 mM threo-Ds-isocitric acid, 1.0 mM NADP+ and 1.0 mM $MnSo_4$ in 50 mM Tris-HCL buffer pH 7.4 at 30 C. The reaction was started with the edition of enzyme and absorbance at 340 nm was noted every 10 second on a Beckman DU- 64 UV-visible spectrophotometer. The initial rate of reaction was determined graphically. One enzyme unit was defined as that amount of enzyme, which brings about the formation of 1 umole of NADPH per minute under the assay conditions.

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* Present address: Department of Veterinary Biochemistry, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Aizwal, Mizoram

The total protein was determined by the method of Lowry et al (1951) using bovine serum albumin as the standard. The specific activity has been expressed in terms of units/mg protein.

Seed germination

Healthy seeds of blackgram with yellowish-green seed coat were selected and soaked in water for 5 hours. After washing several times with tap water, the soaked seeds were spread in a single layer over a wet, properly washed and sterile sand bed covered with a filter paper sheet. The layer of the seeds then covered with a wet filter paper sheet and then allowed to germinate in an incubator adjusted at 27 C. Drying of the sand bed and filter paper sheet was avoided by sprinkling water from time to time during the course of germination.

Enzyme extraction

The $NADP^+$ -dependent isocitrate dehydrogenase was extracted from seeds germinated for different length of time. The germinated seeds were homogenized in 10 mM Tris-HCL buffer pH 7.4 containg 10 mM 2-mercaptoeethanol and 1% (w/v) PVP in a kitchen blende taking 1.5 mL of the buffer for each gram of the germinated seeds. The resulting homogenate was squeezed through a double layer of coarse cotton cloth and then centrifuged in Beckman J2-21 refrigerated high speed centrifuge for 30 minutes at 12,000 g at 4 C. the resulting supernatant was collected as the enzyme extract.

RESULTS AND DISCUSSION

The activity of $NADP^+$ -dependent isocitrate dehydrogenase in the enzyme extract prepared from blackgram seeds germinated at different time periods was found to vary (Fig. 1). The enzyme extract prepared at 0 hr of germination, i.e., immediately after soaking of the seeds in water for 5 hrs was found to have a specific activity (SA) value of 0.02-units/mg protein. The SA of the extract gradually increased till a maximum and then declined. The maximum SA of 0.52 units/mg protein was observed at 48 hrs of germination. Beyond 48 hrs of germination period, the SA gradually declined. At 84 hrs of germination, the SA was only 0.15 units/mg protein. This observation was found to be constant with the findings of Satoh and Nakamura (1984) who determined the SA of $NADP^+$ -dependent isocitrate dehydrogenase in castor bean seeds. The initial increase in SA with the time period of germination may be due to de novo synthesis of the enzyme protein. The gradual decrease in the SA after reaching a maximum level of 0.52 units/mg protein at 48 hrs of germination may be due to inhibition/denaturation of the enzyme by certain metabolites accumulated in the cell during germination. Satoh and Nakamura (1984) also offered similar explanation regarding the decline in the SA of $NADP^+$ -dependent isocitrate dehydrogenase in castor bean.

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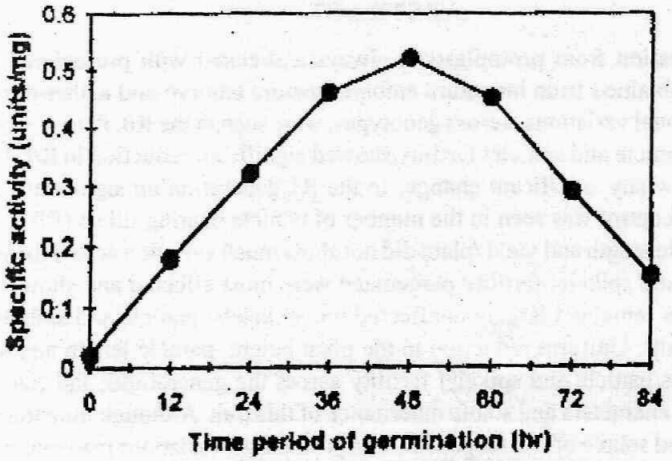

Fig.1: The influence of time periodof germination on the specific activity of $NADP^+$ -dependent isocitrate dehydrogenuse in seeds of blackgram, Vigna murgo (L.) Heppe cv. yellowish green seed coat. Enzyme extraction at each time period of germination was carried out as in Enzyme Extraction taking whole of the germinating seeds obtained from a sample of the dry seeds weighing 50g in total. The specific activity was estimated after determining the enzyme activity adopting the method of Curry and Ting (1976) and the protein according to the method of Lowry et al. (1951)