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Effect of cadmium on activities of some enzymes of glycolysis and pentose phosphate pathway in pea

I.K. CHUGH* and S.K. SAWHNEY

Department of Biochemistry, CCS Haryana Agricultural University, Hisar - 125 004, India

Abstract

Activities of alcohol dehydrogenase, hexokinase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were significantly inhibited by cadmium in germinating pea (*Pisum sativum* L. cv. Bonneville) seeds. The effect was concentration dependent in the range of 0.25 to 1.0 mM $CdCl_2$. The magnitude of detrimental effect on these enzymes was reduced during later stage of germination (9 d) largely because of fall in the activities of these enzymes in the control seeds germinated in water. *In vitro*, activities of hexokinase, glucose-6-phosphate dehydrogenase, and alcohol dehydrogenase were inhibited at 0.5 mM Cd^{2+} in the reaction mixture by 62, 67, and 36 %, respectively, however, 6-phosphogluconate dehydrogenase was insensitive to Cd^{2+}.

Additional key words: alcohol dehydrogenase, germination, glucose-6-phosphate dehydrogenase, hexokinase, 6-phosphogluconate dehydrogenase, *Pisum sativum*.

Introduction

Deleterious effect of Cd^{2+} on photosynthesis (Sawhney *et al.* 1990), respiration (Reese and Roberts 1985, Sawhney *et al.* 1990), nitrogen fixation (Chugh *et al.* 1992), carbohydrate metabolism (Greger and Linderberg 1992), nitrate reduction and ammonia assimilation (Sawhney *et al.* 1990, Chugh *et al.* 1992) are well documented. This metal also interferes with the seed germination and early seedling growth (Soboler *et al.* 1982, Rani *et al.* 1990, Bishnoi *et al.* 1993, Chugh *et al.* 1996). However, investigations on the impact of Cd^{2+} on metabolic reactions in germinating seeds are scanty. Inhibition of mobilization of starch via amylolytic pathway by Cd^{2+} has been reported in germinating pea seeds (Chugh and Sawhney 1996). Content of

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*Address for correspondence: CCS Haryana Agricultural University, Rice Research Station, Kaul-136 021, India; fax: (+91)1662 34952, e-mail: hau@hau.ren.nic.in

several hydrolytic enzymes were found to be low in pigeon pea seeds germinated in presence of Cd^{2+} (Bishnoi *et al.* 1993).

Ripe seeds of pea (Brown 1965) contain only minute quantity of ATP which is just enough to activate/support a few initial physiological processes. Subsequently, the energy requirements of germinating seeds have to be fulfilled through respiration. During early stages of germination, the seed coat and peripheral tissues offer considerable resistance to diffusion of oxygen (Chugh and Sawhney 1996) and the seeds, therefore, are under natural anaerobiosis. Increased activities of enzymes of anaerobic (Cossins *et al.* 1968) and of oxidative degradation of glucose (Nicolus and Aldosoro 1979) in germinating seeds imply their important role in respiratory activities. Any quantitative disturbances in the level of enzyme activities might have profound influence on seedling growth. Therefore, the research was focused on the effects of Cd^{2+} on alcohol dehydrogenase and enzymes of oxidative pentose pathway in germinating pea seeds.

Materials and methods

Germination of seeds: Seeds of pea (*Pisum sativum* L. cv. Bonneville) were pretreated by surface sterilization with 0.2 % mercuric chloride solution for 5 min, rinsed thoroughly with glass distilled water and then placed for germination in filter paper lined Petri plates (15 cm diameter; 30 - 40 seeds in each plate) at 25 °C in dark in an incubator. Each Petri plate had 25 cm^3 of either distilled water (control) or solutions of 0.25, 0.5, or 1.0 mM $CdCl_2$. Streptomycin sulphate (25 $\mu g\ cm^{-3}$) was also included in all solutions to suppress microbial growth. All treatments were replicated twice.

Preparation of cell free extract and enzyme assay: Cell free extract was prepared at 0 - 4 °C. One g of seeds was macerated in 6 cm^3 extraction media in a chilled pestle and mortar. The homogenate was centrifuged at 10 000 *g* for 15 min and the supernatant obtained was used as crude enzyme preparation. Extract was dialysed against the extraction buffer for at least 4 h.

Procedure of Brown and Wray (1968) was followed for extraction and assay of hexokinase (EC 2.7.1.1). Activity of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was computed by subtracting values obtained for 6-phosphogluconate dehydrogenase (EC 1.1.1.47) from those of combined activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Combined activities of these enzymes were determined by the method of Muto and Uritani (1970). For assaying 6-phosphogluconate dehydrogenase, glucose-6-phosphate was omitted from the reaction mixture which was used for assaying combined activities. Alcohol dehydrogenase (EC 1.1.1.1) was extracted and assayed according to Cossins *et al.* (1968). All enzyme assays were conducted on duplicate samples of each treatment and the activity of each extract was determined twice.

Results

Cadmium exerted a marked deleterious effect on activity of alcohol dehydrogenase (Fig. 1*A*). In control seeds, the enzyme activity rose sharply during the first 24 h and then started declining. Similar change in activity of this enzyme was observed in presence of cadmium but its activity was considerably lower. After 36 h of germination, 0.25, 0.5 and 1.0 mM Cd^{2+} diminished the enzyme activity by 10, 35 and 50 %, respectively. Cadmium, at 0.5 mM inhibited *in vitro* activity of alcohol dehydrogenase by 36 % (Table 1).

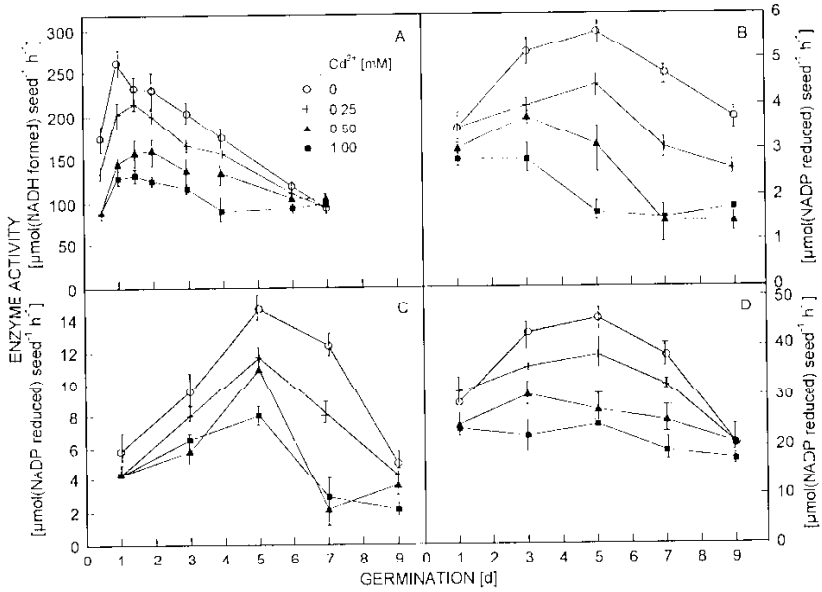

Fig. 1. Effect of 0.25, 0.5, and 1.0 mM $CdCl_2$ on activities of alcohol dehydrogenase (*A*), hexokinase (*B*), glucose-6-phosphate dehydrogenase (*C*) and 6-phosphogluconate dehydrogenase (*D*) in germinating pea seeds.

A substantial activity of hexokinase was detected in seeds germinated in water after 1 d of germination which increased further upto the 5th day and then began to decline (Fig. 1*B*). A quantitatively similar profile occurred in presence of 0.25 mM Cd^{2+} whereas its activity began to decline after 3 d at 0.5 and 1.0 mM Cd^{2+}. On the 5th day, when control seeds exhibited maximum hexokinase activity, 0.25, 0.5 and 1.0 mM Cd^{2+} diminished its activity by 20, 45 and 70 %, respectively. *In vitro* hexokinase activity was inhibited by 62 % in presence of 0.5 mM Cd^{2+} in the reaction mixture (Table 1).

Activity of glucose-6-phosphate dehydrogenase was impaired by 20, 25 and 45 % on 5th day in presence of 0.25, 0.5 and 1.0 mM Cd^{2+}, respectively, which got

accentuated on the 7th day (Fig. 1*C*). The extent of inhibitory effect was much less on the 9th day of germination because of a rapid decrease in the enzyme activity in control. *In vitro* activity of this enzyme was almost equally sensitive as that of hexokinase and was inhibited by about 65 and 80 % in presence of 0.5 and 1.0 mM Cd^{2+} (Table 1).

Table 1. Inhibition [%] of various enzyme activities *in vitro* by cadmium. Enzyme extract was prepared from pea seeds germinated for 3 d in water (control) and the activities were determined in presence of 0.25, 0.5 or 1.0 mM $CdCl_2$ in the reaction mixture.

Enzyme	0.25 mM	0.5 mM	1.0 mM
Hexokinase	58	62	73
Glucose-6-phosphate dehydrogenase	33	67	78
6-Phosphogluconate dehydrogenase	0	0	7
Alcohol dehydrogenase	27	36	16

The activity of 6-phosphogluconate dehydrogenase was also significantly depressed by Cd^{2+} and except for the 9th day remained low as compared to that of control throughout the period of germination (Fig. 1*D*). The effect of Cd^{2+} on this enzyme was concentration dependent. However, unlike glucose-6-phosphate dehydrogenase, *in vitro* activity of 6-phosphogluconate dehydrogenase remained unaffected, even in presence of 1.0 mM Cd^{2+} in the assay mixture (Table 1).

Discussion

In germinating pea seeds glucose is respired predominantly via fermentative reactions (Kolloffel 1967, Raymond *et al.* 1985) and some amount through oxidative pentose phosphate pathway (Nakayama *et al.* 1978, Nicolas and Aldosoro 1979, Reese and Roberts 1985). Metabolism of glucose via either of these pathway requires its prior phosphorylation by hexokinase to glucose-6-phosphate. Activity of this enzyme increased upto 5 d of germination and declined thereafter (Fig. 1*B*). It is also apparent that the activity of hexokinase was significantly depressed by Cd^{2+}. The observation by Reese and Roberts (1985) that in the cell suspension culture of *Nicotiana* raised in Cd^{2+} containing media, glycolysis and oxidative pentose phosphate pathways were affected to almost the same extent is perhaps accounted by diminished activity of hexokinase which supplies the common substrate for both these pathways. The rapid increase in activity of alcohol dehydrogenase during initial stages of germination followed by gradual decline (Fig. 1*A*) is consistent with its role in fermentative respiration and is in agreement with the observed changes in its profile accompanied by the reported accumulation of ethanol in seeds by the earlier worker (Cossins *et al.* 1968, Suzuki and Kyuwa 1972, Beratini *et al.* 1980). Cd^{2+} exerted a deleterious effect on activity of alcohol dehydrogenase (Fig. 1*A*). Enhancement of activity of alcohol dehydrogenase involves activation of the enzyme molecules already present in

cotyledons of germinating pea seeds (Cossins *et al.* 1968, Suzuki and Kyuwa 1972). Transition of the inactive enzyme is associated with change of disulfide to dithiols (Suzuki and Kyuwa 1972). Hence, the depressed activity of alcohol dehydrogenase appears to be due to interference of Cd^{2+} with the disulfide-dithiol transition mechanism. The possibilities that the heavy metal might be inhibiting the activated form of enzyme by interacting with other sulfhydryl groups (Cossins *et al.* 1968) or imidazole group of a histidine residue (Stiborová and Doubravová 1987) cannot, however, be ruled out.

Unlike hexokinase maximum activities of NAD-glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase were detected 1 d after germination and throughout the experimental period their activities were not affected at all by Cd^{2+} (data not shown). Interestingly, *in vitro* activity of glyceraldehyde-3-phosphate dehydrogenase was also found to be insensitive to Cd^{2+} (data not shown). Whereas hexokinase determines the availability of glucose-6-phosphate for respiration as well as the other cellular processes, alcohol dehydrogenase plays a crucial role of ensuring sustained supply of NAD needed for activity of triose-phosphate dehydrogenase. Although Cd^{2+} did not exert any discernible effect on activities of the examined enzymes of glycolysis, NAD-glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase, the catabolism of glucose via this pathway would be impeded due to decreased level of the hexokinase and alcohol dehydrogenase.

Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are the two important enzymes of oxidative pentose phosphate pathway. The former catalyses the initial and regulatory reaction and, therefore, controls the metabolism of glucose-6-phosphate via this pathway. In agreement with the results of Brown and Wray (1968), the control seeds attained maximum activity of both the enzymes after 5 d of germination followed by a decline in their activities (Fig. 1*C,D*). Increase in activities of these enzymes during germination in other leguminous seeds has been reported (Cherry 1963, Nicolus and Aldosoro 1979). Cd^{2+} exerted a profound deleterious effect on activities of these enzymes in germinating pea seeds (Fig. 1*C,D*). In contrast Van Assche *et al.* (1988) reported enhanced activity of glucose-6-phosphate dehydrogenase in leaves of Cd^{2+} treated seedlings of *Phaseolus vulgaris*. In their studies activities of malic enzyme and NADP-isocitrate dehydrogenase in the leaves were also promoted. Such a response of these NADPH generating enzymes was considered to be physiologically important for ensuring adequate supply of NADPH even under the conditions when the Hill activity is inhibited by the heavy metal. It is noteworthy that *in vitro* activity of glucose-6-phosphate dehydrogenase was inhibited by the Cd^{2+} while that of 6-phosphogluconate dehydrogenase was virtually insensitive. Inhibition of *in vitro* activity of glucose-6-phosphate dehydrogenase in leaf extracts of *Silene cucubalus* was also reported by Mathys (1975). Lower activity of glucose-6-phosphate dehydrogenase in germinating seeds could either be due to its inactivation and/or depressed formation. The impact of Cd^{2+} on 6-phosphogluconate dehydrogenase is most likely to be through suppression of its synthesis. In this context it is relevant that the appearance of combined activities of these two enzymes in germinating pea seeds is prevented by actinomycin D (Brown and Wray 1968), thus denoting their *de novo* synthesis.

Cd^{2+} is likely to suppress the operation of oxidative pentose phosphate pathway due to diminished availability of glucose-6-phosphate as well as because of its adverse impact on the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Inhibition of fermentative reaction, which predominates during early stages of germination, would impose severe handicap right from the beginning with respect to generation of ATP. This would hamper the synthesis of nucleic acid, proteins, lipids, *etc.* (Mayer and Poljakoff-Mayber 1989). Bulk of NADPH needed for biosynthetic reaction is produced via oxidative pentose phosphate pathway. Oxidative pentose phosphate pathway also performs another vital function of providing D-ribose needed for production of nucleotides and consequently of nucleic acids. Apparently Cd^{2+} by interfering with this pathway would curtail the capacity of the germinating seeds to synthesize nucleotides as well as nucleic acids. All the enumerated changes would lead to drastic reduction in the growth of the seedlings.

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