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The role of sulphur in detoxication of cadmium in young sugar beet plants

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Abstract

In young sugar beet plants cadmium suppressed the activity of nitrate reductase, glutamine synthetase and glutamate dehydrogenase, whereas sulphur exhibited a protective role towards activity of these enzymes, except of glutamine synthetase. Protein synthesis was suppressed in the absence of S in nutrient medium; the lowest level was at 10^{-3} M Cd^{2+}. Chloroplast pigment contents were increased by S while Cd^{2+}, even in the lowest concentration, (10^{-5} M) showed a repressive effect. The highest concentrations of Cd^{2+} (10^{-3} M) caused a decrease in dry mass, whereas S induced its increase. Nitrate content was increased in the presence of Cd^{2+} and decreased by increased concentration of S.

Additional key words: *Beta vulgaris* L., carotenoids, chlorophyll, glutamate dehydrogenase, glutamine synthetase, nitrate reductase, protein biosynthesis.

Introduction

The increasing interest in environmental pollution has led to investigation of Cd^{2+} uptake, its distribution in different plant species (Jarvis *et al.* 1976), and its effect on plant metabolism. Plant metabolism may be affected by Cd^{2+} in different ways. Cadmium is an effective inhibitor of chlorophyll biosynthesis (Stobart *et al.* 1985), photosynthesis (Weigel 1985), respiration and the activities of several enzymes (Lee *et al.* 1976, Bishnoi *et al.* 1993). In winter wheat (*Triticum aestivum* L. cv. HV 8) Cd^{2+} caused a growth retardation and changes in ion uptake. Immediate target of Cd^{2+} is the cell membrane, where both the membrane composition and function can be altered and damaged (Fodor *et al.* 1994).

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There are several ways by which the plants can reduce these negative effects of Cd^{2+}. One of the possibilities, and this hypothesis has recently become very popular, is that heavy metals form chelates with the sulphur-rich proteins (Wagner 1984). Besides, sulphur is a structural constituent of amino acids and several coenzymes and prostetic groups, such as ferredoxine, which are important for nitrogen assimilation (Petrović and Kastori 1994).

In view of the above we investigated the cadmium effect on protein biosynthesis and activity of nitrogen metabolism enzymes: nitrate reductase (NR), glutamine synthetase (GS) and glutamate dehydrogenase (GDH) in young sugar beet plants grown under conditions of different supply of sulphur.

Materials and methods

The experiments were conducted in a greenhouse on young plants of sugar beet (*Beta vulgaris* L.), hybrid NS-Hy 11. After germination of seeds in vermiculite, the plants were grown in 1/2 Hoagland's nutrient medium of the following composition [mM]: 2.5 $Ca(NO_3)_2$; 2.5 KNO_3; 1.0 KH_2PO_4; 1.0 $MgSO_4{\cdot}H_2O$ and [µM]: 23.1 B; 4.6 Mn; 0.38 Zn; 0.052 Mo; 0.16 Cu and 8.95 Fe(III)Na EDTA. After 21 d the plants were divided into three groups, and the plants of each group were grown in nutrient solutions containing different sulphur concentration: without S (control), 1.0 and 3.0 mM of S. Two weeks after, the plants from all three variants of S nutrition were grown in the media with different concentrations of Cd^{2+}: without Cd^{2+} (control), 0.01 and 1.0 mM of Cd^{2+}. The plants were taken for experiments after 7 d of treatment by various concentrations of Cd^{2+}.

The contents of chlorophylls *a* and *b*, and carotenoids were determined spectrophotometrically in the acetone extract of freshly harvested leaves, using molar extinction coefficients according to Holm (1954) and Wettstein (1957).

Nitrate reductase activity was determined in leaves by an *in vivo* method, in phosphate buffer, pH 7.4 (Hageman and Reed 1980). The activities of the *in vitro* transferase reaction of GS and NADH-dependent GDH were determined in a common extract from leaves according to the method described by Coombs and Hall (1982). The extract was prepared by homogenization of 1.0 g of fresh leaves in 10 cm^3 of extraction buffer (containing 50 mM imidazole, 5 mM 2-mercaptoethanol and 0.5 mM EDTA, pH 7.2), followed by centrifugation at 10 000 *g* for 15 min.

Plant proteins were prepared by extraction with trichloroacetic acid in acetone according to Damerval *et al.* (1986). Electrophoresis was carried out on 10 % SDS-polyacrylamide gel containing 0.1 % SDS with 4.5 % stacking gel. 50 µg of proteins were applied on each lane. Densitometric analysis of stained gels was carried out by *ISCO gel scanner*, equipped with a *ISCO Type 6* optical unit. Data were integrated using a *Hewlett Packard 3396 II* integrator.

Results and discussion

Sulphur causes a significant increase in the NR activity (Fig. 1A): at 1 and 3 mM S the NR activity was increased 4.5 and 6.6 times, respectively, compared to the control. At the same time, Cd^{2+} suppressed the NR activity: at 1 mM S and the Cd^{2+} concentration of 0.01 and 1.0 mM the respective activities were 44.9 % and 26.2 % of that for plants grown at 1 mM S without Cd^{2+}. At 3 mM S, the respective NR activities were 45.4 % and 36.1 %. These data indicate that S exhibited a protective role by lowering the toxic effect of Cd^{2+} on the activity of NR.

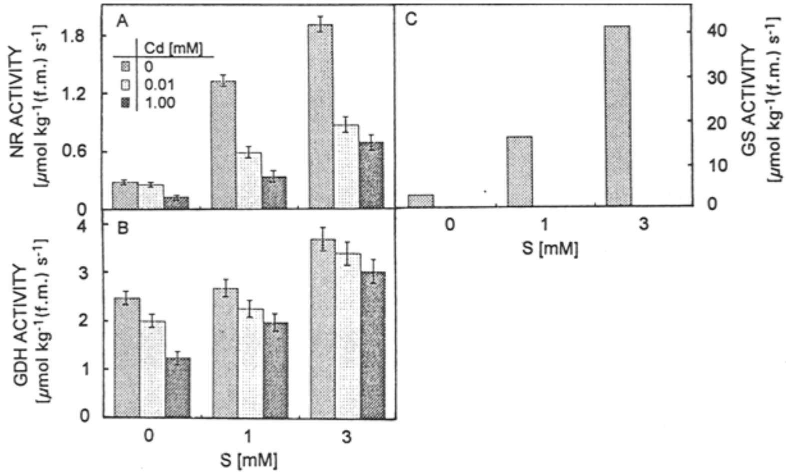

Fig. 1. Effect of Cd concentration on nitrogen metabolism enzymes activity in young sugar beet plants differently supplied with sulphur. *A* - nitrate reductase activity, *B* - glutamine synthetase activity, *C* - glutamate dehydrogenase activity.

In contrast to this, Cd^{2+} caused a total inhibition of GS activity in leaves of young plants of sugar beet, and no protective effect of sulphur was observed (Fig. 1C).

The presence of S caused a substantial increase in GDH activity compared to the control and the increase was 8.5 and 49.8 % in the presence of 1 and 3 mM S, respectively. The presence of Cd^{2+} caused a significant suppression of GDH activity compared to the control (plants grown without S and Cd^{2+}), so that the measured activities were 80.8 % and 49.9 % for 0.01 and 1.0 mM Cd^{2+}, respectively. In plants grown on the medium containing S, the presence of Cd^{2+} caused a smaller, but still significant, decrease of GDH activity (Fig. 1B). At 1 mM S and 0.01 or 1.0 mM Cd^{2+} this activity was 84.3 % or 73.8 % of the control activity, respectively. In the presence of 3 mM S the decreases in GDH activity were lower, so that 92.4 % and 82.4 % of the control activity were obtained for 0.01 and 1.0 mM Cd^{2+}, respectively. Therefore, as in the case of NR, S plays the protective role of GDH.

Our results, as well as the results of other studies, indicate that the activities of enzymes depend upon the presence of heavy metals and their concentrations. It was found that Cu^{2+}, Hg^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, *etc.* strongly inhibit the rice root GS because of their reaction with the thiol groups in the enzyme (Miflin and Lea

1980). Kastori *et al.* (1993) found that the activities of NR, GS and GDH and the content of soluble proteins were highest in young sugar beet plants grown on the medium with 10^{-5} M Fe. Both iron deficiency (10^{-7} M Fe) and its excess (10^{-3} M Fe) tended to depress activities of these enzymes, as well as the content of soluble proteins. The absence of S, however, did not affect significantly GDH (Friedrich and Schrader 1978). At the same time, these authors found an increased nitrate content in plants grown in the absence of S. However, Sasakawa and Yamamoto (1977) found that the shortage of S caused no significant effect either on the NR, NiR, GDH activities, or nitrate content.

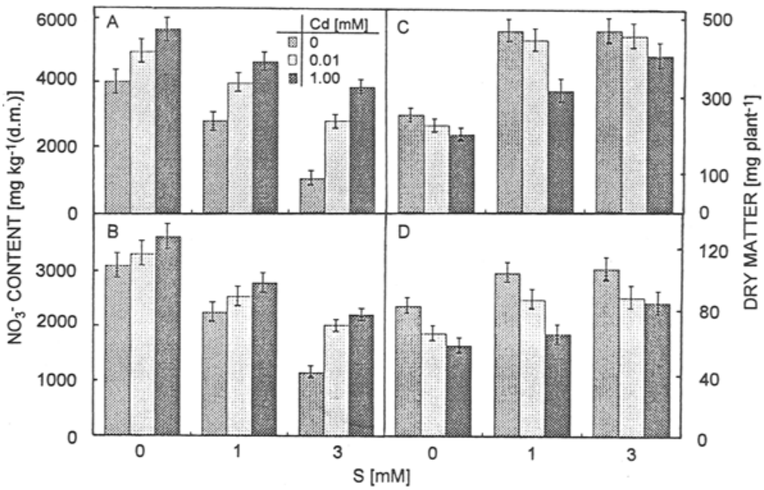

Fig. 2. Effect of Cd concentration on content of NO_3^- and dry matter in young sugar beet plants differently supplied with sulphur. *A* - NO_3^- content - above ground part; *B* - NO_3^- content - roots; *C* - dry matter content - above ground part; *D* - dry matter content - roots.

Table 1. Protein fractions [% of total protein]

Fraction on the gel	Sulphur concentration [mM]	Cadmium concentration [mM] 0	0.01	1.0
	0	1.9	0.7	-
Fraction A	1	21.1	21.4	17.8
	3	21.9	19.8	20.6
	0	4.4	3.0	-
Fraction B	1	3.7	4.0	4.1
	3	3.3	3.5	4.0
	0	4.2	4.0	-
Fraction C	1	6.6	8.9	6.0
	3	7.9	9.6	4.6

Presence of Cd^{2+} exhibited a negative effect on the nitrogen assimilation enzymes. As a consequence, the nitrate contents in both the above-ground part and root of the investigated plants were increased (Fig. 2A and B). Thus the highest nitrate content was observed in plants grown in the absence of S. Therefore, the nitrate content and the investigated S concentrations are in a negative correlation, so that the lowest nitrate content is observed at 3 mM S. The favourable effect of S on the nitrate content is best noticeable at the highest Cd^{2+} concentration.

In the absence of S the protein synthesis was suppressed (Fig. 3, lanes 1, 4 and 7) and showed the lowest level at 1 mM Cd^{2+} (lane 1). Presence of 1 mM S maintained protein synthesis practically at the control level (lanes 2, 5 and 8) with a slightly lower effect at 1 mM Cd^{2+}. 3 mM S showed a smaller effect (lanes 6 and 9) except at 1 mM Cd^{2+} (lane 3).

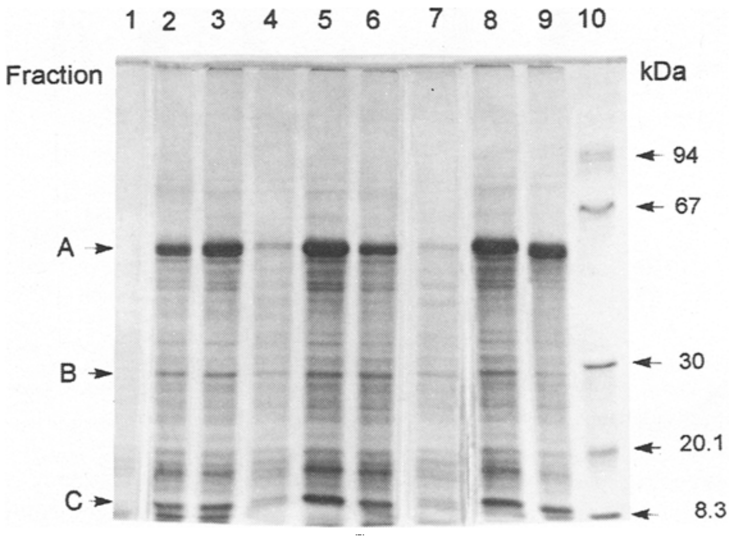

Fig. 3. SDS-PAGE patterns of proteins from leaves of sugar beet young plants grown on nutrient media with different supply of sulphur and cadmium. Lanes: 1 - NMWS/1 mM Cd; 2 - 1 mM S/ 1 mM Cd; 3 - 3 mM S/1 mM Cd; 4 - NMWS/0.01 mM Cd; 5 - 1 mM S/0.01 mM Cd; 6 - 3 mM S/0.01 mM Cd; 7 - NMWS/NM; 8 - 1 mM S/NM; 9 - 3 mM S/NM; 10 - marker proteins. (NMWS - nutrient medium without S; NM - nutrient medium)

The densitometric measurements of gels showed an increase of the portion of lower molecular mass protein fractions (about Mr = 10 000) with the increase in S concentration. According to Wagner (1984) Mr of these fractions corresponds to Mr of cadmium-binding proteins, which could mean that the presence of S yields and increase in relative contents of proteins which are responsible for its detoxication. Also, the relative content of the most abundant fraction on the gel (it can be supposed that it is a RUBISCO protein) decreases with an increase Cd^{2+} concentration, both at the optimal and enhanced provision of plant in S. However, the ratio of this fraction at 1 mM Cd^{2+} is by 16 % higher than in the case of 3 mM S and approaches the corresponding value observed with control plants. This indicates that S plays a protective role towards this fraction (Table 1).

Increase in S concentrations caused a significant increase in dry mass content of both the above-ground part and root of investigated plants. However, the dry matter content and the investigated Cd^{2+} concentrations are in a negative correlation. In view of this, the lowest dry matter content was found in plants grown in the absence of S and at 1 mM Cd^{2+} (Fig. 2C and D). At the same time, the obtained results indicate that the unfavourable effect of Cd^{2+} on dry matter content was significantly counteracted by S present at an optimal or a luxurious level.

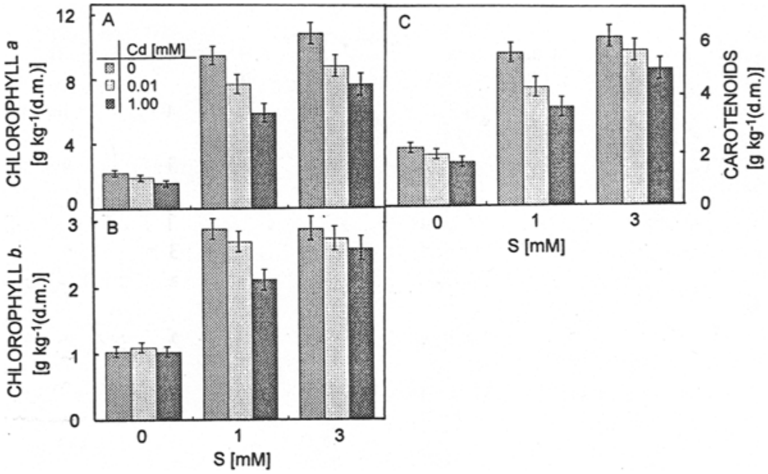

Fig. 4. Effect of Cd concentration on content of chloroplast pigments in young sugar beet plants differently supplied with sulphur. *A* - chlorophyll *a* content; *B* - chlorophyll *b* content.

As could be expected, the investigated factors exhibited similar tendency on the contents of chloroplast pigments. The highest changes were noticed in the content of chlorophyll *a*, then chlorophyll *b*, and the lowest in total carotenoids (Fig. 4).

The obtained results show that the presence of S in the nutrient medium decreased the harmful effect of cadmium on all the investigated parameters except of GS.

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