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PHOSPHOLIPID METABOLISM IN ROOTS AND MICROSOMES OF SUNFLOWER SEEDLINGS: INHIBITION OF CHOLINE PHOSPHOTRANSFERASE ACTIVITY BY BORON*

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Key Word Index—*Helianthus annuus*; Compositae; Sunflower; roots; microsomes; lipids; choline phosphotransferase; boron.

Abstract—The action of boron on phospholipid composition and synthesis in roots and microsomes from sunflower seedlings has been studied. The fatty acid composition and relative amounts of individual molecular species of phospholipids in roots and microsomes were very similar. In both the content of phospholipids was decreased and the relative levels of their component fatty acids changed by treatment with 50 ppm of boron. This concentration of boron in the culture medium was found to inhibit the *in vivo* [1-^{14}C] acetate incorporation into root lipids and that of [Me-^{14}C] choline into phosphatidylcholine of root microsomes. Cytidine-5-diphospho (CDP)-[Me-^{14}C] choline incorporation into phosphatidylcholine of isolated microsomes was also inhibited by 50 ppm of boron when present in the growth medium of seedlings. These results indicate that the decrease in phosphatidylcholine labelling from [^{14}C] choline observed when root microsomes were treated with boron would be caused by a decrease in CDP-choline phosphotransferase activity.

INTRODUCTION

Several studies have demonstrated a close relationship between lipid composition of different higher plants and deficiencies in and toxicity of mineral nutrients, e.g. changes in lipid composition in wheat and oat roots as a function of Ca^{2+} and Mg^{2+} concentration [1], a general decrease of all lipids and an increase of the lipid-unsaturation in plants subjected to saline stress [2, 3], and inhibition of phospholipid synthesis by Ca^{2+} in roots of calcicolous plants [4, 5]. In relation to boron, Shkol'nik and Kopman [6] showed that in sunflower plants a deficiency of this element provoked a decrease in the phospholipid content. More recently, work carried out in our laboratory has demonstrated that media with a high B concentration induced in sunflower cotyledons a decrease in the degradation rate of triacylglycerols, an increase in free fatty acid content and an inhibition of lipoxygenase activity [7].

These facts and the more recent hypotheses on the effects of boron in plants, which indicate an action of this micronutrient on membranes [8–10], have prompted the present work on the composition and biosynthesis of phospholipids in roots and microsomes of sunflower seedlings.

RESULTS

The phospholipid composition of 10 and 50 ppm boron-treated whole tissues and microsomes is shown in Table 1; phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the most abundant compounds. In both, 50 ppm of boron induced a marked decrease in phospholipid contents. Likewise, the relative amounts of fatty acids in phospholipids of microsomes were changed, with 18:2 being increased upon treatment with 50 ppm of boron (Table 2).

[1-^{14}C] Acetate incorporation into the total lipids of sunflower roots, expressed as nmol of labelled substrate incorporated per 100 roots, is higher in tissues maintained for three days in media with 10 ppm than in media with 50 ppm of boron (Table 3). ^{14}C is incorporated, mainly, into neutral lipids but also into PE and 50 ppm of boron stimulated the biosynthesis of TAG but reduced that of PC (Table 4).

The *in vivo* incorporation of [Me-^{14}C] choline into total acyl-lipids of microsomes isolated from roots developed in media with 10 or 50 ppm of boron takes place almost entirely into PC (Table 5). A concentration of 50 ppm of boron induced a decrease in the biosynthesis of this phospholipid relative to microsomes isolated from roots grown in media with 10 ppm of boron. In the same way, after incubating the microsomes purified from these tissues with cytidine 5-diphospho (CDP)-[Me-^{14}C] choline, almost all the radioactivity incorporated into total lipids is to be found in PC (data not shown). The enzymatic incorporation of CDP-[Me-^{14}C] choline into PC with incubation time is shown in Fig. 1. The activity was linear during the first 60 min in microsomes isolated from roots grown in media with 10 ppm of boron. However, in membranes from tissues grown in 50 ppm of boron, a much stronger inhibition of activity was observed. When ^{14}C incorporation is measured in terms of the protein concentration, it is also higher in microsomes prepared from roots grown in 10 ppm of boron than in those isolated from roots developed in 50 ppm of boron,

* Part 6 in the series 'The functions of boron in plants'. For Part 5 see ref. [14].

Table 1. Phospholipid composition of roots and microsomes from sunflower seedlings developed for three days in media with 10 or 50 ppm of boron

Phospholipids	Whole roots		Microsomes	
	10 ppm	50 ppm	10 ppm	50 ppm
PI	177.8 ± 23.1 (12.3)	162.0 ± 23.7 (14.3)	36.0 ± 1.8 (12.7)	13.3 ± 1.0 (11.4)
PC	737.4 ± 88.2 (51.3)	581.7 ± 65.2 (51.3)	125.6 ± 8.7 (44.4)	47.3 ± 4.3 (40.5)
PE	373.4 ± 41.0 (25.9)	224.1 ± 27.0 (19.8)	95.6 ± 4.8 (33.8)	42.4 ± 2.9 (36.3)
PA	151.1 ± 19.3 (10.5)	165.5 ± 18.1 (14.6)	25.5 ± 4.8 (9.1)	13.8 ± 0.8 (11.8)
Total	1439.7	1133.3	282.7	116.8

All experiments were performed in triplicate and the results, expressed as percentages (figures in brackets) and in μg per 100 roots (whole tissues) or per mg of proteins (microsomes), are means ± S.D..

PI, Phosphoatidylinositol; PC, phosphatidylocholine; PE, phosphatidyl-ethanolamine; PA, phosphatidic acid.

Table 2. Acyl-phospholipid composition of roots and microsomes from sunflower seedlings developed for three days in media with 10 or 50 ppm of boron

		Acyl lipids					
		PC		PE		PA	
Fatty acids		Roots	Microsomes	Roots	Microsomes	Roots	Microsomes
16:0	10 ppm	26.9	36.2	37.8	39.7	34.9	35.1
	50 ppm	30.9	26.1	32.0	35.1	31.1	26.0
18:0	10 ppm	2.8	2.7	4.0	2.5	7.9	4.4
	50 ppm	3.6	1.7	2.0	1.4	7.8	1.7
18:1	10 ppm	7.6	7.1	8.2	3.4	15.0	7.8
	50 ppm	3.2	4.8	17.9	2.4	12.9	4.9
18:2	10 ppm	55.1	45.8	46.9	48.9	37.4	46.2
	50 ppm	55.0	59.2	43.1	56.6	42.5	61.6
18:3	10 ppm	7.6	8.2	3.1	5.5	4.7	6.5
	50 ppm	7.3	8.1	5.0	4.4	5.7	5.7

Data given as percentages of total fatty acids are the average of three independent experiments, not differing by more than 10% from the mean. n.d., not detected.

Table 3. *In vivo* [1-^{14}C] acetate uptake into sunflower roots developed for three days in media with 10 or 50 ppm of boron and its incorporation into their total lipids

Incubation medium (added nmol)		Uptake (% added nmol)	Total lipids (% incorporation)	(nmol/10 roots)
34.5	10 ppm	6.91 ± 0.86	73.52 ± 7.03	1.75 ± 0.21
	50 ppm	5.16 ± 1.23	39.41 ± 8.21	0.70 ± 0.17

The experiments were performed in triplicate and the results are means ± s.d.

Table 4. *In vivo* [1-^{14}C] acetate incorporation into different acyl-lipids of sunflower roots developed for three days in media with 10 or 50 ppm of boron

Boron treatments	[1-^{14}C] Acetate incorporation into acyl-lipids, %						
	TAG	MAG + DAG + FFA	PI	PC	PE	PA	Other
10 ppm	11.3 ± 1.5	22.5 ± 5.5	5.1 ± 0.7	34.5 ± 3.5	18.0 ± 2.5	4.2 ± 0.5	4.3 ± 0.6
50 "	16.8 ± 2.0	21.6 ± 3.4	6.1 ± 0.9	25.5 ± 2.8	20.2 ± 2.4	4.9 ± 0.6	4.9 ± 0.9

The experiments were performed in triplicate and the results, expressed as a percentage of acetate incorporated into each acyl-lipid, are means ± s.d.

TAG, triacylglycerols; MAG, monoacylglycerols; DAG, diacylglycerols; FFA, free fatty acids.

Table 5. *In vivo* [Me-^{14}C] choline uptake into sunflower roots developed for three days in media with 10 or 50 ppm of boron, and into total lipids and phosphatidylcholine of their *microsomes*

Incubation medium (added nmol)	^{14}C-Choline incorporation into microsomal acyl-lipids		
	Uptake (% added nmol)	Total lipids (nmol/mg of proteins)	Phosphatidylcholine (nmol/mg of proteins)
10 ppm 5.2	66.6 ± 7.9	1.64 ± 0.25	1.61 ± 0.29
50 ppm	61.0 ± 8.5	1.13 ± 0.18	1.11 ± 0.19

Each value is the average of two independent experiments ± s.d.

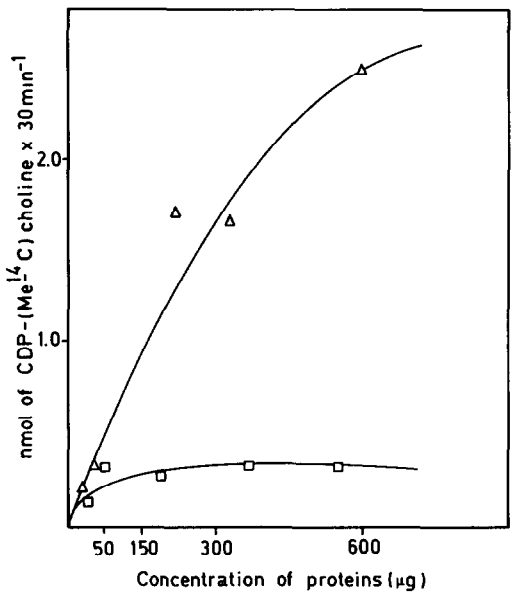

Fig. 1. Effect of incubation time on phosphatidylcholine synthesis from CDP-[Me-^{14}C] choline by isolated microsomes from sunflower roots developed in media with 10 (△) or 50 ppm (□) of boron. Each value is the average of two independent experiments, not differing by more than 12% from the mean. Results in nmol of CDP-[Me-^{14}C] choline incorporated into phosphatidylcholine per mg of proteins.

where the activity reaches a maximum with very low microsomal protein concentration (Fig. 2).

The choline phosphotransferase activity exhibited normal Michaelis–Menten kinetics when the concentration of the substrate CDP-[Me-^{14}C] choline was varied. The double reciprocal plot of velocity with CDP-[Me-^{14}C] choline as the variable substrate allowed the determination of an apparent K_m of 7.8 μM, for the enzyme of microsomes from roots developed in 10 ppm of boron, or 6.8 μM, for the enzyme of microsomes from tissues grown in 50 ppm of boron, while the V_{max} was 6.7 times greater for the enzyme of microsomes isolated from sunflower roots developed in 10 ppm than in 50 ppm of boron (Fig. 3).

DISCUSSION

To estimate the effect of boron on phospholipid metabolism in root membranes, we used a microsomal fraction to compare the phospholipid composition and biosynthesis in a cell-free preparation to that in the whole tissue. Our results show how the phospholipid composition of sunflower seedling roots is similar to that of roots of other higher plants [5, 11]. The results derived from the phospholipid analysis of the microsomal fraction reflect the composition of the whole tissue. In relation to the action of boron on phospholipid components, these results suggest a more active formation of cellular membranes in microsomes isolated from roots grown in 10 ppm of boron. In the same way, the increase in the 18:2

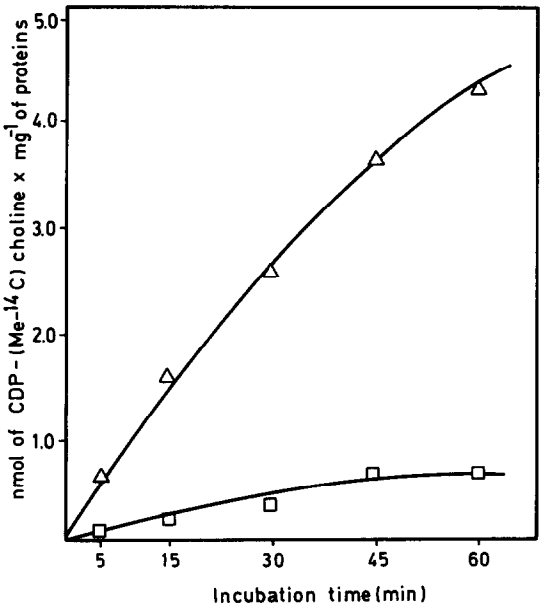

Fig. 2. Effect of increasing concentration of proteins of isolated microsomes from sunflower roots developed in media with 10 (△) or 50 ppm (□) of boron on CDP-[Me-^{14}C] choline incorporation into phosphatidylcholine. Each value is the average of two independent experiments, not differing by more than 15% from the mean. Results in nmol of CDP-[Me-^{14}C] choline incorporated into phosphatidylcholine per 30 min.

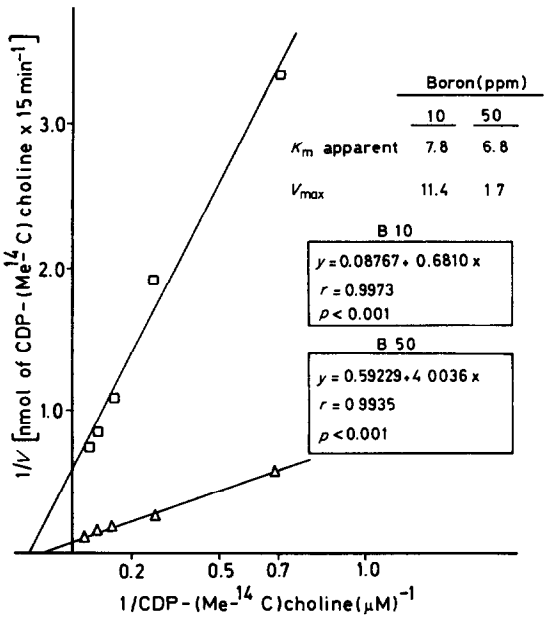

Fig. 3. Lineweaver–Burk plots of the rate of phosphatidylcholine synthesis against CDP-[Me-^{14}C] choline concentration by CDP-choline; 1,2-diglyceride-choline phosphotransferase of microsomes isolated from sunflower roots developed in media with 10 (△) or 50 ppm (□) of boron.

content of different microsome phospholipids induced by 50 ppm of boron, could indicate an effect of this micronutrient on fatty acid desaturase enzymes.

The changes observed in the phospholipid content of whole roots and in their isolated microsomes as a function of boron concentration in the culture medium of the seedlings, led us to study the action of the element on the evolution of lipid synthesis and CDP-choline phosphotransferase activity. Taking into account that the fresh weight of the roots used in our experiments did not change very much with the concentration of boron in the medium (166 ± 13 mg fr. wt per 10 roots, for 10 ppm of boron, and 132 ± 12 mg, for 50 ppm of boron), it is possible to suggest that a lower *in vivo* incorporation of [1-^{14}C] acetate and [Me-^{14}C] choline in to the lipids of whole roots and microsomes maintained in 50 ppm of boron, could be due to an inhibition of lipid synthesis and not to factors derived from differences in the growth of the tissues.

On the other hand, the inhibition of PC biosynthesis in microsomes from roots grown in 50 ppm of boron, suggests changes in the metabolism of this phospholipid at the cellular membrane levels because of the toxicity of this element. Hirsch and Torrey [12] have already demonstrated by means of ultrastructural studies that a boron deficiency condition in sunflower plants leads to a rapid loss of the integrity of the membranes. Previously, Shkol'nik and Kopman [6] had shown in the same plants subjected to the same conditions of deficiency a decrease in phospholipid content.

The above results led us to study, in microsomes, the effect of boron concentration on the activity and some properties of the enzyme CDP-choline: 1,2-diacylglycerol-choline phosphotransferase, which is involved in PC synthesis, a phospholipid associated in plants and animals with the endoplasmatic reticulum [13]. The concentration of boron in the growth medium does not affect the apparent K_m for CDP-[Me-^{14}C] choline, whilst the apparent V_{max} changes from 11.4 nmol of CDP-[Me-^{14}C] choline incorporated per 15 min, in the case of 10 ppm of boron, to 1.7, in the case of 50 ppm of boron. It is also apparent from the K_m values that the concentration of CDP-[Me-^{14}C] choline routinely employed in our experiments (2.4 μM) is not in the substrate-saturation range.

Our results suggest that the decrease in PC labelling from [^{14}C] choline obtained when root microsomes were treated with 50 ppm of boron would be caused by a decrease in CDP-choline phosphotransferase activity. Thus, it is possible to conclude that there exists a relationship between the concentration of boron in the culture medium and the phospholipid metabolism in roots and microsomes of sunflower seedlings.

EXPERIMENTAL

Materials. Sunflower seeds (*Helianthus annuus* L.) used were of the variety HK; the conditions of culture are described in ref. [14]. Three days after transfer of the etiolated seedlings to a medium containing 0.2 mM $CaSO_4$ and 10 or 50 ppm of boron, roots were detached. Sodium [1-^{14}C] acetate (sp. act. 58 mCi/mmol), [Me-^{14}C] choline chloride (sp. act. 58 mCi/mmol) and cytidine 5-diphospho-[Me-^{14}C] choline (sp. act. 56 mCi/mmol) were purchased from Amersham International.

Isolation of root microsomes. Crude microsomes were prepared from 3-day-old roots (10 g fr. wt) developed in a medium with 10

or 50 ppm of boron. Roots were homogenized in a mortar with the following medium (1:2 w/v, roots: soln): 100 mM Tris–HCl buffer, pH 7.5, containing 0.4 M sucrose, 10 mM $MgCl_2$, 10 mM KCl, 1 mM EDTA, 2 mM DTT, 3 mM cysteine and 1.5% PVP. The homogenate was filtered through four layers of nylon cloth and centrifuged at 10000 *g* for 15 min; the supernatant was again centrifuged at 105000 *g* for 60 min, the supernatant discarded and the pellet resuspended in 1 ml of the previous soln without PVP using a Potter homogenizer. The crude microsome prepn was further purified by sucrose gradient centrifugation at 27000 *g* for 60 min, in a discontinuous gradient prepared by layering 4 ml each of 68, 65, 58, 55, 46, 41 and 34 sucrose conc. % w/w, from the bottom to the top of the tube, in 50 mM Tris–HCl buffer, pH 7.5, 10 mM KH_2PO_4, 1 mM KCl and 0.1% BSA. The bands were sepd and, after dil to 0.4 M sucrose with the buffer soln, were centrifuged at 105000 *g* for 30 min. The pellet was collected and the NADH-cytochrome c-reductase activity insensitive to antymicin. A associated with the microsomes tested by the method described in ref. [15]. The band between 48 and 55% sucrose concn showed the greatest enzyme activity.

Lipid analysis. Total lipids were ext either from the whole roots or from their isolated microsomes by homogenization in $CHCl_3$–MeOH–H_2O according to ref.[16]. The $CHCl_3$ phase was taken to dryness and the residue redissolved in 1 ml C_6H_6–EtOH (4:1). Lipids were sepd by TLC on silica gel using the systems described in refs [17, 18]. Fatty acid Me esters from individual lipids were prepared according to ref. [19] and analysed by GC using Me heptadecanoate as int st.

In vivo *synthesis of root and microsome lipids.* Three-day-old roots were taken from seedlings grown in 10 or 50 ppm of B and carefully washed. The 10 roots used in each expt were incubated in the dark for 3 hr at 28° with either 2 μCi of Na [1-^{14}C] acetate, to label the root lipids, or with 0.3 μCi of [Me^{-14}C] choline chloride, to label microsomal PC. After incubation, roots were washed to remove adhering medium and the lipids extd. Total lipids were fractionated by TLC and determined by liquid scintillation counting.

In vitro *synthesis of microsome PC*. Microsomes isolated from root seedlings grown in media with 10 or 50 ppm of B were fed with 0.2 μCi of cytidine 5-diphospho [Me-^{14}C] choline in the dark for 30 min at 28° under continuous agitation. The incubation medium contained in a final vol of 1.5 ml, 15 μmol Tris–HCl, pH 8, 10 μmol $MgCl_2$, 20 μmol DTT, 5 μmmol dipalmitin (added as an emulsion in 0.3% w/v of Tween 80) and membrane proteins (0.5 mg). Before the addition of the enzyme prepn, the reaction mixt. was preincubated for 5 min. After incubation, the reaction was stopped by heating the mixture with 1 ml MeOH at 90° for 5 min. Lipids were extd and analysed as described above. Activity of choline phosphotransferase was determined as nmoles of labelled substrate incorporated into PC/mg proteins/hr.

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