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Boron is required for the stimulation of the ferricyanide-induced proton release by auxins in suspension-cultured cells of *Daucus carota* and *Lycopersicon esculentum*

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Boron deficiency reduces the ferricyanide-induced net proton release of suspension-cultured carrot (*Daucus carota* L.) and tomato (*Lycopersicon esculentum* Mill.) cells by more than 50%. This effect is reversed within 60 to 90 min by the addition of B. Vanadate (400 μM) completely suppresses the proton release, indicating an ATPase-driven process. The differences between B treatments do not appear when auxins are omitted from the experimental solution, but can be observed within less than 30 min after the addition of auxin to auxin-deficient cell cultures. This suggests, that an adequate supply of B is required for the auxin action to take place.
The results are discussed with respect to the primary functions of B in membranes and transport processes, and its possible influence on auxin-induced metabolic events.

Key words – ATPase, boric acid, boron, carrot, cell cultures, *Daucus carota*, 2,4-dichlorophenoxyacetic acid, ferricyanide, indole-3-acetic acid, iron, *Lycopersicon esculentum*, potassium-hexacyanoferrate(III), proton release, tomato, vanadate.

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Introduction

It has frequently been shown that B plays a role in the uptake and translocation of nutritional elements (e.g. Alexander 1942, Mitchell et al. 1953, Pollard et al. 1977, Rehm 1937, Robertson and Loughman 1973, 1974, Smyth and Dugger 1980). The true nature of this regulatory function, however, still remains obscure.

We have reported the effects of B supply on membrane potential and ferricyanide-induced proton release (Blaser-Grill et al. 1989). We present results of further experiments on interactions between B and factors which influence the proton release of higher plant cells.

Abbreviations – 2,4-D, 2,4-dichlorophenoxyacetic acid; DES, diethylstilbestrol; IAA, indole-3-acetic acid; DCCD, dicyclohexylcarbodiimide; NPA, naphthylphthalamic acid; TIBA, 2,3,5-triiodobenzoic acid.

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Materials and methods

Cell cultures

Carrot (*Daucus carota* L.) and tomato (*Lycopersicon esculentum* Mill.) cell cultures were grown in the dark in a modified Murashige and Skoog medium (Seitz and Richter 1970). The cell cultures were obtained from stocks maintained at the Univ. of Tübingen, and the Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Munich, FRG.

Experimental set-up

This was essentially as described earlier (Blaser-Grill et al. 1989). In most of the experiments, cells were kept for 16 h under B-deficient conditions. Usually, B deficiency affected the ferricyanide-induced proton release between < 1 h and up to 6 h after transfer to B-deficient

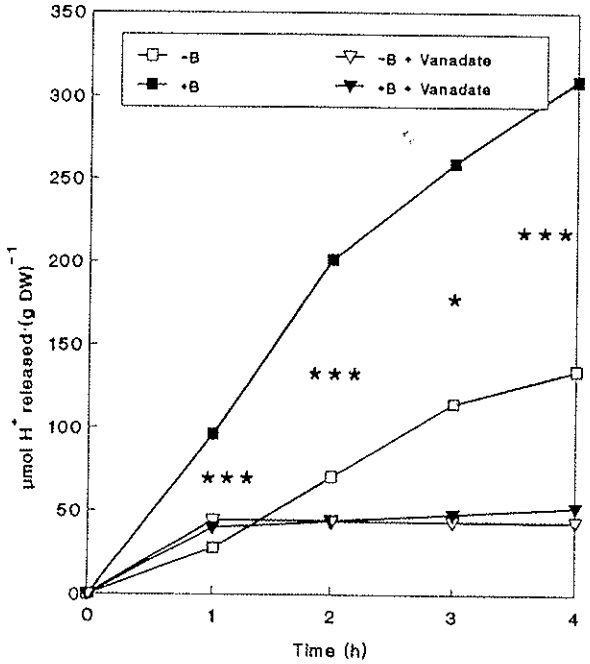

Fig. 1. Net proton release of suspension-cultured carrot cells induced by 1 m*M* $K_3Fe(CN)_6$ during 4 h as influenced by B-deficiency (pretreatment for 16 h with or without 0.1 m*M* H_3BO_3) and 400 μ*M* $NaVO_3$ (supplied at t = 0); *, ***, indicates proton release rates significantly different at the 5% or 0.1 % level, respectively, according to *t*-test between +B and −B treatments; statistical treatment was not applied to the vanadate treatments; +B = 0.1 m*M* H_3BO_3).

conditions, although the magnitude of the initial response varied between individual experiments. We thus chose a longer pretreatment period to get more uniform results.

Before the start of the experiment, cells were separated from their culture medium by gentle vacuum filtration and repeated rinsing with an essentially B-free nutrient solution. The stimulation of net proton release was induced by the addition of ferricyanide [$K_3Fe(CN)_6$] at the beginning of the experiment (t = 0). The pH was measured with an Orion-Ross combination electrode and a Beckman model Φ11 pH-meter. All experiments were carried out under sterile conditions in a laminar flow cabinet. Between individual measurements, the flasks with cell suspensions were kept on a gyratory shaker at 105 rpm. The individual concentrations of auxin, ferricyanide, and boric acid additions are indicated in the figures.

Statistical evaluation

Experiments were statistically evaluated by *F*- and *t*-testing either by the computer-program SAS (SAS Inst. Inc., NC, USA) or MS AVA 16 (Bayerische Landwirtschaftsministerium). Statistical treatment has been applied to the proton release rates (i.e. ΔH^+ (g fresh or dry weight)$^{-1}$ Δt^{-1}). However, as cumulative data are more illustrative, they have been given in the Figs 1 and 2. Stars and arrows indicate proton release rates that are significantly different. pH values of replicates usually did not differ by more than ± 0.05 units. Experiments were run with 4–6 replicates, and each experiment reported has been run at least thrice with similar results. Data from typical experiments are reported below.

Results

In cell cultures of tomato or carrot, a net proton release can be induced by omitting iron (Blaser-Grill et al. 1989) or by supplying ferricyanide (Fig. 1). In general, tomato and carrot cells behaved similarly, but more experiments were run with carrot cells as they are easier to handle. In B-deficient cultures, the proton release is reduced by ca 50% (or even more) over a 3–4 h period. This reduction can be reversed within 30–90 min after adding B again (Fig. 2). Vanadate at 400 μ*M* inhibited the proton release completely in both B-deficient and B-sufficient cultures (Fig. 1). The concentration of vanadate did not kill the cells during the experimental period, although a lower concentration of 100–200 μ*M* would have been sufficient to inhibit the plasmalemma ATPase, as seen in a follow-up experiment.

When testing a range of ferricyanide and auxin concentrations, the net proton release was more inhibited by B deficiency at the higher concentrations of auxin (2-

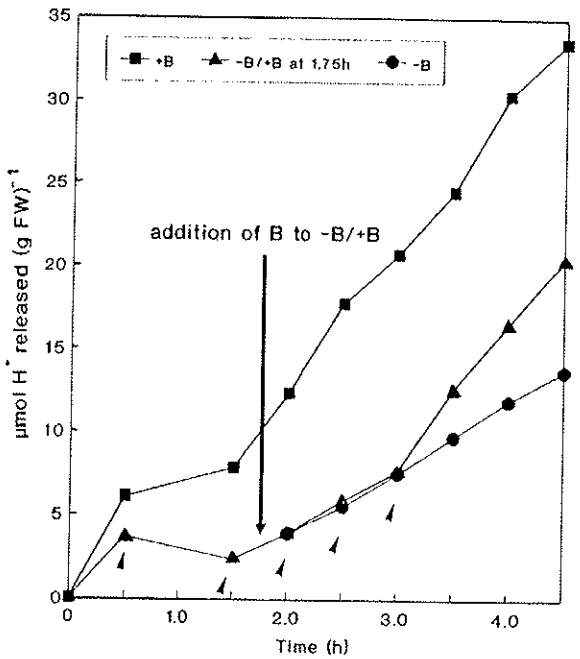

Fig. 2. Influence of B resupply (0.1 m*M* H_3BO_3 at t = 105 min) to B-deficient carrot cell cultures on proton release induced by 0.5 m*M* ferricyanide; continuous B-supply (0.1 m*M*) = +B, B (0.1 m*M*) supplied at 1.75 h = −B/+B; −B shows the average net proton release under B-deficiency as calculated from another experiment; small arrows, proton release rates significantly different ($P \leq 0.05$) between +B and −B/+B.

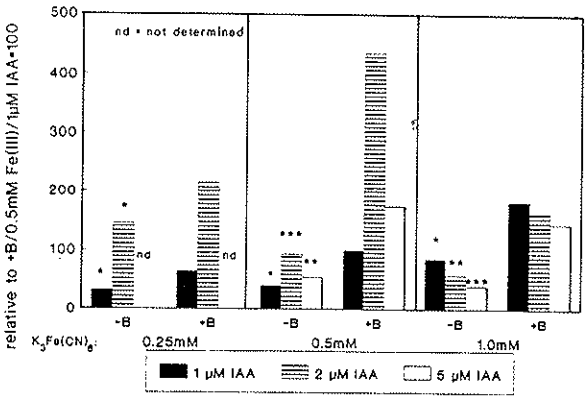

Fig. 3. Influence of ferricyanide and IAA concentrations on the net proton release by suspension-cultured carrot cells precultivated with 0.1 m*M* H_3BO_3 (+B) or without B-supply (−B) for 16.5 h; *,**,***, net proton release rates significantly different between B-treatments at the 5, 1 or 0.1% level, respectively.

and 5-fold compared to the normal nutrient solution), although the proton release rate was somewhat inhibited by 5 µ*M* IAA (Fig. 3). The maximum of net proton release was obtained at a ferricyanide concentration of 0.5 m*M* and 2 µ*M* IAA.

Preculturing auxin-heterotrophic tomato cell cultures without auxin to exhaust their auxin reserves and running the experiment without the addition of auxin (2,4-D), led to a lower net proton release rate with no difference between B treatments (Fig. 4, first two sets of columns). This effect was observed in all experiments which were carried out in this way. Usually, net proton release in B-deficient cultures was slightly higher (though not always significantly) than in B-sufficient ones. Upon the addition of auxin (2,4-D), there was a sudden increase in proton release within less than 30 min, which was much more pronounced in the B-sufficient cultures (Fig. 4, columns at the right side).

Discussion

As vanadate completely eliminates the Fe-deficiency- or ferricyanide-induced stimulation of proton release (Fig. 1), the participation of a process driven directly or indirectly by ATPase is indicated (recent preliminary experiments showed a strong inhibition by DCCD, and slightly by DES). This is in agreement with the observations of Römheld et al. (1984) for Fe-efficient plants. In auxin-heterotrophic cell cultures, the mechanism obviously depends to a large extent on an adequate supply of auxins. As shown above (Figs 2 and 4), B as boric acid or borate is required for the action of auxins, at least in dicotyledonous cells (in maize cell cultures, we have not obtained significant responses so far). Interactions between boron and auxins have been reported recently (Jarvis et al. 1984, Tang and DelaFuente 1986a,b). We have found indications for a reduced translocation of abscisic acid and glucose (Fackler et al. 1985, Goldbach 1985). According to Borkovec and Procházka (1989) these processes can also be stimulated by IAA.

The mechanism(s) by which B enables auxins to act is not yet identified. Although Jarvis et al. (1984) assume two independent processes for the action of B and IAA, our results point to a closer relationship. There are several possibilities for an interaction of B with auxins:

1. B might stabilise the plasma membrane in such a way, that auxins can be bound to possible receptor sites. The stabilising action of B on membranes may take place through the formation of boric acid or borate-esters with hydroxyl groups of membrane molecules such as glycoproteins or glycolipids. A stabilising action of boron on membranes has been suspected by several authors (Parr and Loughman 1983, Pollard et al. 1977, Smyth and Dugger 1980). It can not be excluded, however, that B interacts directly with auxin receptor molecules. Löbler et al. (1987) identified a glycoprotein with 15–20 sugar residues as an auxin-binding polypeptide. The sugar residues could offer binding sites for boric acid/borate. The ferricyanide-induced proton relase could be completely inhibited by the auxin-inhibitor TIBA, but not by NPA. (H. Goldbach, T. Rötzer and M. Porzelt, unpublished results).
2. Boron-deficiency rapidly reduces protein synthesis (Hundt et al. 1970), probably by inhibiting DNA synthesis (Krueger et al. 1987). Krueger et al. (1987) (and references therein) assume a reduced rate of purine or pyrimidine nucleotide supply as the cause of the observed reduction in DNA synthesis. The biochemical mechanism, by which B might affect this process, remains unclear, especially when considering the low concentrations of B in the cytoplasm.
3. The signal transduction from the receptor-bound auxin to protein syntesis is thought to be mediated by second messengers such as Ca^{2+}/calmodulin (Evans

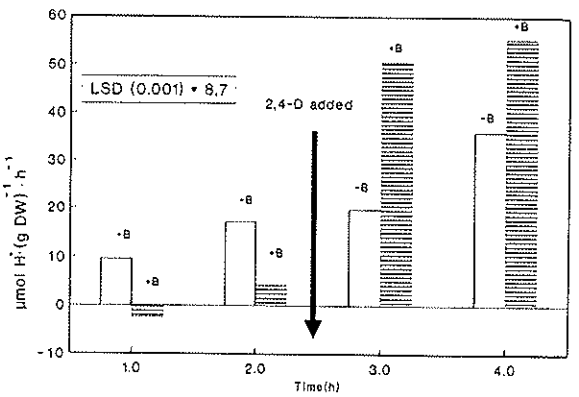

Fig. 4. Ferricyanide-induced net proton release as influenced by adding 0.1 µ*M* 2,4-D after 2.5 h to auxin-starved tomato cell cultures, kept either with 0.1 m*M* H_3BO_3 (= +B) or without B (= −B) for 16 h; the columns indicate the proton release rate per unit of time and dry weight.

et al. 1987) and/or inositol phosphates (Berridge and Irvine 1989, see also review of Poovaiah et al. 1987). B might therefore be a factor in controlling these mechanisms rather than the auxin-binding or DNA-synthesis itself.

There is evidence, that auxins stimulate protein synthesis (Borkovec and Procházka 1989, and references therein) by activating a few specific genes (see Key 1987). The rapid synthesis of certain proteins might also be responsible for further medium- and/or long-term increases in overall protein synthesis. It thus seems logical to assume that B influences the protein synthesis by modifying the cell's response to auxins, e.g. by affecting processes involved in the signal transduction from auxin uptake or binding to the induction of specific genes rather than by participating directly in the process of transcription or translation or the supply of nucleotide precursors. Taking into account the delay in reaction after adding B to B-deficient cells (usually 60–90 min), B should control other processes than the binding of auxins just by changing the membrane configuration, as this should yield a more rapid response. The proton release, however, increased much faster upon the addition of auxins than after the addition of B (in the presence of auxins). This clearly points to an influence of B on processes somewhere in between the binding of auxins and the final response.

To summarize, B might influence uptake and translocation of (nutrient) elements within the plants as well as their reaction to environmental stimuli (see e.g. Tanada 1978) by modifying the plants response to auxins. This helps to explain a variety of different responses to B deficiency in the literature (Tanada 1974, 1978, see also reviews by Dugger 1983, Pilbeam and Kirkby 1983, Shkolnik 1984). It seems possible that B affects the auxin action by influencing mechanisms related to signal transduction, which might be even relevant to animal metabolism (see also Weser 1968).

Further experiments with different inhibitors of protein synthesis as well as with different Ca^{2+} and auxin antagonists are under way, and should help in further elucidation of the primary function(s) of B in cell metabolism.

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