UPTAKE AND ASSIMILATION OF AMMONIUM IONS BY CUCUMBER SEEDLINGS FROM SOLUTIONS WITH DIFFERENT pH AND ADDITION OF HEAVY METALS

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ABSTRACT

Influence of Cu^{2+} , Cd^{2+} , Pb^{2+} and Fe^{2+} in various pH conditions (5.0, 6.0, 7.0) on the uptake and assimilation of NH_4^+ by cucumber seedlings was estimated. Every metal in different pH of uptake solution distinctly reduced ammonium absorption calculated from NH_4^+ depletion. Copper and ferric ions, but not cadmium or lead, astonishingly decreased the uptake of ammonium from solution at pH 5.0. Cu^{2+} was also very active at pH 6.0. The accumulation of ammonium in roots of metals-treated seedlings at the same time was high. The high level of ammonium in root cells despite of its low uptake probably resulted from disturbance in NH_4^+ assimilation. Both glutamine synthetase (GS) and NADH-glutamate dehydrogenase (NADH-GDH) – the major enzymes in ammonium assimilation, were inhibited after one hour of plant exposition to the metals. Similarly as in the case of ammonium uptake, the influence of pH was visible only in combination with copper and ferric ions. The strongest reduction of enzyme activities was observed for Cu^{2+} and Fe^{2+} in pH 5.0 and 6.0. The various metals absorption by roots from solutions with different pH was not detected. The data show correlation between metal inhibition of GS and NADH-GDH activities and metal inhibition of ammonium absorption.

KEY WORDS: Ammonium uptake and assimilation, heavy metals, glutamine synthetase, NADH-glutamate dehydrogenase, pH.

INTRODUCTION

The mechanism of NH_4^+ transport into plant cells is still poorly understood. Early studies of Haynes and Goh (1978) and Clarkson (1979) indicated that the net ammonium uptake was not directly dependent on the metabolic activity of roots. For this reason authors suggested the passive character of ion transport. Recently, Wang et al. (1993) showed that the mechanism of ammonium transport into cells depends on the external NH_4^+ concentration. Ammonium uptake at concentration above 1 mM is mediated by linear low-affinity transport system and is passive. When external NH_4^+ concentration drops below 1 mM, ammonium is taken up actively by saturable system with high affinity to substrate. The driving force for an active ammonium uptake is the proton electrochemical gradient across the plasma membrane (Glass 1988). It is well established that the electrochemical gradient is created by plasma membrane H^+ -ATPase. On the other hand the rate of ammonium absorption depends on ammonium assimilation, as a free NH_4^+ accumulates in plant and its conversion to NH_3 can dissipate transmembrane proton gradients as well as inhibit activity of different enzymes required in many metabolic processes (Bloom 1988).

In the present work we have examined the influence of metals on ammonium uptake and accumulation and on GS and NADH-GDH activity. Different pH of uptake solution was tested since various pH determined both, ammonium uptake

and metals mobilities. The cucumber seedlings were used, since studies of heavy metal influence on NO_3^- uptake and on the activity of plasmalemma H^+ -ATPase and oxidoreductase, have been performed in the same plants, using similar exposition times and similar concentrations of metals (Burzyński and Buczek 1994a and b).

MATERIAL AND METHODS

Plant material

The cucumber seedlings were cultivated as in earlier work (Burzyński and Buczek 1997). After germination seedlings were grown hydroponically in nutrient solution without nitrogen for 4 days, (for nutrient solution composition see Burzyński and Buczek 1997). The pH was adjusted to 6.0. Plants were grown in a growth chamber with a light-dark period of 17h/7h and 25°C/20°C under artificial lamps 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Ammonium uptake and accumulation

Groups of 20 four-day-old seedlings were placed in 100 cm^3 of uptake solution containing: 100 μM $(\text{NH}_4)_2\text{SO}_4$ and heavy metals as CuCl_2 (100 μM), CdCl_2 (100 μM), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (100 μM) or PbCl_2 (500 μM) in 0.33 mM Mes-NaOH buffer pH: 5.0, 6.0 or 7.0. The concentration of metals was chosen on the basis of earlier works (Burzyński and Buczek 1994a and b). The solutions with different pH (5.0, 6.0, 7.0)

without metals were used as a control. In pH 7.0 Pb partially precipitated, but despite of that, all measurements in this combination were made. Uptake solutions were continuously mixed and aerated and after two hours, the uptake and accumulation of NH_4^+ were determined. The level of ammonium in external solution and in hot water extracts from plants tissue was measured colorimetrically according to Hecht and Mohr (1990).

Enzyme activity

Glutamine synthetase (GS) was assayed after Hipkin and Syrett (1977) and the activity of NADH-glutamate dehydrogenase (NADH-GDH) was measured according to Duke *et al.* (1975). Enzymes activities were measured after one hour of plant exposure to ammonium and heavy metals in Mes-NaOH buffers (pH 5.0, 6.0, 7.0). All values presented in the paper are representative for the results obtained in five independent experiments.

Heavy metals

The level of Cu, Pb, Cd and Fe was determined using atomic absorption spectrophotometer (Perkin Elmer model 3300). Heavy metals were measured immediately in the uptake solution at the beginning and after one hour of plant exposition. In plant material the investigated metals were measured after digesting of dried plant material in 63% HNO_3 , in microwave sample preparation system (MDS-2000).

RESULTS AND DISCUSSION

After 2 hours of plant exposure to Fe, Cd, Cu, or Pb, ammonium uptake was significantly reduced (Fig. 1). Decrease in ammonium absorption can be a result of NH_4^+ influx depression or/and enhancement of NH_4^+ efflux. The reduction of net ammonium uptake was most visible in plants treated with copper and iron at pH 5.0 (18 and 5 per cent of control, respectively) and copper at pH 6.0 (15% of control). Cu in pH 7.0, Fe in pH 6.0 and 7.0 and Cd and Pb in every investi-

gated pH decreased NH_4^+ absorption to 50-70%. Pb despite of partial precipitation in pH 7.0 was similarly active in this pH as in pH 5.0 and 6.0. Concentration of ammonium at the beginning of the experiment was 200 μM , so according to Wang *et al.* (1993) and Wang *et al.* (1994) the absorption of NH_4^+ from such concentration is energy-dependent and proton electrochemical gradient across the plasma membrane is necessary. Heavy metals like Cu, Cd, Pb, Hg, Ni and Zn in various degrees may interact with membrane components. The plasma membrane ATPase and oxidoreductase responsible for electrogenic transport are the potential sites of interaction of metal ions (Kennedy and Gonsalves 1989, Ros *et al.* 1992, Burzyński and Buczek 1994b). The results of metal action were reduction of trans-root potential and H^+ -ions efflux (Kennedy and Gonsalves 1987 and 1989). Influence of iron on the plasma membrane composition and polarization was not investigated, but a high iron internal content caused a lot of similar symptoms as in the case of other heavy metals. (Bode *et al.* 1995). The better transport and mobility of Cu and Fe from a more acidic solution should be the simplest explanation of different action of Cu and Fe according to pH of uptake solution. However the level of metal in solutions after one hour of experiment decreased in every pH about 60-80 percent for Cu, Cd and Pb and almost 100% for Fe (Fig. 2). Taking together, it is clear that adsorption and/or uptake of Cu and Fe by roots was irrespective of acidity of rhizosphere. There still remains an open question whether Cu and Fe from acidic external solution were faster transported across the cell wall and across the plasma membrane or/and in acidic environment Cu and Fe create the chemical forms which are more toxic and more bioavailable. Despite low NH_4^+ absorption by plants treated with metals, especially with Cu in pH 5.0 and 6.0 and Fe in pH 5.0, accumulation of ammonium in root cells was high (Fig. 3). Higher level of free NH_4^+ in maize plants which were grown in nutrient with excess of Cu, Mn or Zn was also observed by Dhillon *et al.* (1986). Low absorption and high accumulation of ammonium ions indicate the reduced rate of ammonium assimilation. Primary NH_4^+ assimilation takes place in root cells (Oaks 1994). It is now generally accepted that the principal pathway of ammonium

Fig. 1. The ammonium absorption by plants exposed to Fe, Cd, Cu, or Pb in different pH of uptake solution. Groups of twenty cucumber seedlings were placed in the 100 cm³ of ammonium uptake solutions containing: 100 μM $(\text{NH}_4)_2\text{SO}_4$ and 100 μM , FeCl_2 , CdCl_2 , CuCl_2 or 500 μM PbCl_2 in 0.33 mM Mes-NaOH buffer. pH was adjusted to: 5.0, 6.0 or 7.0. After 2 hours depletion of ammonium from uptake solution was measured. Means values $\pm$ SE from 10 repetition are given.

Fig. 2. The influence of pH of uptake solution on Fe, Cd, Cu, and Pb absorption by roots of cucumber seedlings. Absorption of the metals was calculated from ions depletion in the uptake solution after one hour of experiments. The results are shown as percent of all amount of metal supplied into uptake solution. Additional explanations as in Fig. 1.

Fig. 3. The accumulation of NH_4^+ in cucumber roots treated with Fe, Cd, Cu or Pb in different pH of uptake solution. Explanations as in Fig. 1

assimilation in higher plants involves the action of two enzymes: glutamine synthetase (GS) and glutamate synthase (GOGAT), and that glutamate dehydrogenase (GDH) plays only a small role in ammonium assimilation. However this two competing enzymes (GS and GDH) are spatially separated within cell and NH_4^+ level in mitochondria is high enough to support a GDH action (Oaks and Hirel 1985 and references therein). Translocation of metals to hypocotyls and cotyledons was insignificant after one hour (Fig. 4). Therefore the influence of the metals on glutamine synthetase and glutamate dehydrogenase was measured only in roots. Activities of GS, as well as GDH were reduced after one-hour of plant exposure to metals in all combinations (Figs 5 and 6). These results indicate that the influence of investigated metals on ammonium absorption could depend not only on the changes in plasmalemma property but also on the metal action on ammonium assimilation. Interesting in our results is that similarly as with ammonium absorption, the degree of toxic action of Cu and Fe on enzymes activities depended on pH of uptake solution (Figs 5 and 6). In pH 5.0 and 6.0 Cu decreased GS activity to 22% and 32% and GDH to 10 and 18 percent of control respectively. In pH 7.0 repression of GS activity was only to 60% and GDH to 40%. GS activity after plant exposure to Fe at pH 5.0 was reduced to 45 and GDH to 40 percent of control, in combination with Fe in pH 7.0 the ac-

Fig. 5. The glutamine synthetase (GS) activity in roots after one hour of seedlings treatment with investigated metals in different pH. Explanations as in Fig. 1.

Fig. 6. The NADH-glutamate dehydrogenase (GDH) activity in roots after one hour of seedlings treatment with Fe, Cd, Cu or Pb in various pH of uptake solution. Composition of uptake solution and concentration of metals as in material and methods.

vities of both enzymes were almost as in the control. Thus, the acidity of external solution changed the toxic action of Cu and Fe not only on ammonium absorption, but also on the activities of major enzymes in NH_4^+ assimilation. Further in-

Fig. 4. The accumulation of Fe, Cd, Cu and Pb in organs of cucumber seedlings after 0.5, 15, 30 and 60 minutes of plant exposure to the metals. Plants were put into the uptake solution (see Material and methods). pH 6.0. After 5, 15, 30 and 60 minutes roots were rinsed with distilled water, and the level of metal was measured separately in dried roots, hypocotyls and cotyledons.

vestigations are necessary to explain the reason of this correlation.

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POBIERANIE I ASYMLACJA JONÓW AMONOWYCH Z ROZTWORÓW O RÓŻNYM pH Z DODATKIEM METALI CIĘŻKICH (Cu, Cd, Pb, Fe) PRZEZ SIEWKI OGÓRKA

STRESZCZENIE

Badano wpływ Cu^{2+} , Cd^{2+} , Pb^{2+} i Fe^{2+} na pobieranie i asymilację jonów amonowych przez siewki ogórka przy zastosowaniu roztworu zewnętrznego o różnym pH (5.0, 6.0, 7.0). Wszystkie badane metale niezależnie od pH roztworu redukowały absorpcję amonu. Jony miedzi i żelaza w odróżnieniu do jonów ołowiu i kadmu szczególnie mocno hamowały pobieranie NH_4^+ z roztworu o pH 5.0. Miedź była również aktywna w przypadku pH 6.0. Równocześnie mimo obniżonego pobierania amonu stwierdzono wysoką akumulację NH_4^+ w korzeniach siewek traktowanych metalami. Wysoki poziom amonu w tkance przy niskim tempie jego pobierania można tłumaczyć zaburzeniami w asymilacji pobranego NH_4^+ . Wykazano, że oba enzymy zarówno syntetaza glutaminowa (GS) jak i NADH-dehydrogenaza glutaminianowa, czołowe enzymy biorące udział w asymilacji amonu, już po jednej godzinie traktowania roślin metalami miały dużo niższą aktywność niż enzymy z kombinacji kontrolnej. Podobnie jak w przypadku pobierania amonu wpływ pH na działanie metali na enzymy uwidocznił się w przypadku miedzi i żelaza. Wyraźnie silniejsze działanie inhibujące Cu i Fe na aktywność obu enzymów stwierdzono dla pH roztworu zewnętrznego równego 5.0 i 6.0. Mierzono absorpcję metali poprzez ich ubytek z roztworów ale nie stwierdzono by zależna ona była od pH roztworu zewnętrznego. Wyniki wskazują na wyraźną korelację pomiędzy inhibicją przez metale absorpcji amonu a inhibicją aktywności enzymów asymilujących ten kation.

SŁOWA KLUCZOWE: Pobieranie i asymilacja amonu, pH, metale ciężkie syntetaza glutaminowa, NADH-dehydrogenaza glutaminianowa.