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Changes in glutamine synthetase activity in presence of aluminium complexes

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ABSTRACT In acidic soil release of aluminium (Al) from the solid phase in the rhizosphere is a stress for plants. The pH strongly influences speciation of Al(III), leading to distinct changes in Al phytotoxicity. A hypothesised mechanism of Al tolerance is the chelation and detoxification of Al(III) by organic acids. Internal or external complex formation determines the behaviour of Al in the cell. Some natural and synthetic compounds were tested in glutamine synthetase (GS, EC 6.3.1.2) assay. GS (total) was extracted from leaves and roots of common wheat (*Triticum aestivum* L.) grown hydroponically. Magnesium activates both form of GS, but the enzyme activity shows characteristic changes along the Mg^{2+} concentration range (0-32 mM). Our results confirmed the well-known protective role of citrate and malate, but presented a group of Al(III)-complexes, which activated the GS. In this group the ligand itself had no effect on reaction, however the Al(III)-complex enhanced the GS activity, even in low-Mg^{2+} samples. Despite of this stimulation, Al^{3+} could not substitute Mg^{2+} in the suboptimal Mg^{2+}-range, and it was not a competitor either. **Acta Biol Szeged 46(3-4):103-104 (2002)**

KEY WORDS

aluminium
complex
glutamine synthetase
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Acidification of soil in the rhizosphere and subsequent release of aluminium (Al) from the solid phase is a stress for plants. The pH strongly influences speciation of Al(III), small pH changes in the pH range between 3 and 5 can alter the Al(III) species in solution leading to distinct changes in Al phytotoxicity. Long-term exposure of plants to Al(III) inhibits the growth via induction of nutrient (Ca, Mg, P) deficiencies (Godbold and Jeschke 1998). Al(III) readily enters the symplast of root cells. Aluminium has been reported to inhibit the Mg^{2+}-dependent, K^+-stimulated ATPase in the plasma membrane, affects protein conformation, ion uptake/efflux, phytosiderophore secretion, binds to nucleic acids leading to harmful changes in metabolism (Zsoldos et al. 1999). A hypothesised mechanism of Al tolerance is the chelation and detoxification of Al(III) by organic acids. Internal or external (root exudates) complex formation determines the behaviour of Al in the cell (Ma and Hiradate 2000).

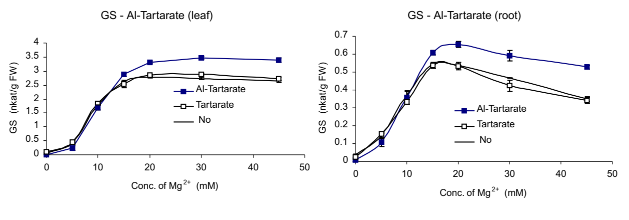

Figure 1. Al(III)-tartarate 1:3 complex represents the first group. Here belongs lactate, saccharate and imino-diacetate (IDA). The common feature of these compounds, that Al-complex has a moderate activation effect, (even in the suboptimal Mg-range), the ligant itself is neutral: no Mg-binding, no change in GS activity.

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In higher plants, glutamine synthetase (GS, EC 6.3.1.2) is responsible for the primary assimilation of ammonium originating from soil or generated in dinitrogen fixation and nitrate reduction. In addition, GS is also involved in the cellular detoxification of the ammonium released in many metabolic processes such as photorespiration or proteolytic degradation (Lea et al. 1990). GS is an octameric enzyme, containes bound Mg^{2+} in its structure. Mg^{2+} is essential for the activity. In our experiments the effect of organic Al(III)-complexes were tested in vitro, on GS activity.

Materials and Methods

Organs (leaves, roots) of 7-day-old wheat (*Triticum aestivum* L.), grown hydroponically in complete nutrient solution were used as source of glutamine synthetase (GS, EC 6.3.1.2). GS was measured in vitro ("synthetase" reaction), according to the colorimetric assay of Rhodes et al. (1975), with slight modification. Native PAGE was performed according Laemmli (1970).

Results and Discussion

Thousands of potential ligands for Al^{3+} are available in the living organisms. Some natural (lactate, tartarate, oxalate, malonate, malate, citrate, saccharate) and synthetic compounds (IDA, NTA, NTA3p, EDTA, [8 mM]) were tested in glutamine synthetase assay. GS (total) was extracted from leaves and roots of common wheat. Magnesium activates both form of GS, but the enzyme activity shows characteristic changes along the Mg^{2+} concentration range (0-32 mM) (see control curves on Figs. 1, 2 and 3).

Our results confirmed the protective role of citrate and malate (Fig. 2), but presented groups of Al(III)-complexes,

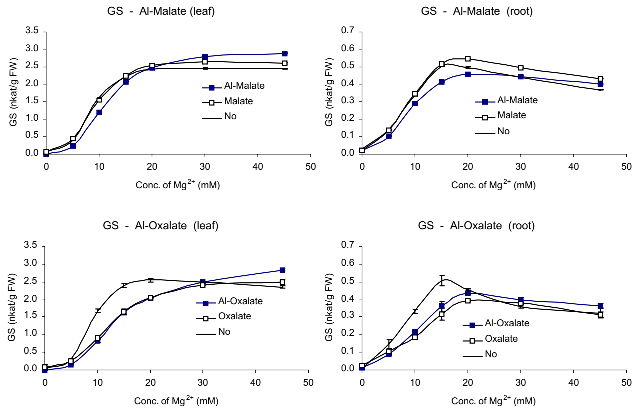

Figure 2. Al(III)malate and Al(III)oxalate (malonate, citrate complexes too) form the group of natural "tolerance"-ligands. The complexes have no activation ability, the ligands slightly bind magnesium, because of this the curves are shifted to the right.

which were active even at pH 7.4. Lactate complex has only a moderate activation ability (Fig. 1). In the third group the ligand itself had no effect on reaction, however the Al(III)-complex enhanced the GS activity (in the case of Al(III)NTA near 100% increase), even in low-Mg^{2+} samples (Fig. 3). Despite of this stimulation, Al^{3+} could not substitute Mg^{2+} in the suboptimal Mg^{2+}-range and it was not competitor either. The effect of Al(III)-complexes was never inhibitory. With some ligands (*e.g.* NTA) the effect of Al(III)-complex was not identical on GS of root or leaf origin (GS_1, GS_2). Due to this and the similar ion radius of Al^{3+}, but higher charge density, and thus stronger Lewis-acidity than that of Mg^{2+}, some specific and direct action of the Al(III)-complexes on the enzyme is supposed including conformational changes, *e.g.* on the Mg^{2+} binding site. The effect is similar to the activation of bovine erythrocyte acetylcholinesterase, which is explained by an interaction between Al^{3+} and ?-peripherial site of enzyme, leading to conformational change and raised activity (Zatta et al. 1994). Some Al-complexes increased the v_{max} of active GS forms and activated inactive form(s) of GS revealed by activity staining of the gel following a native PAGE separation.

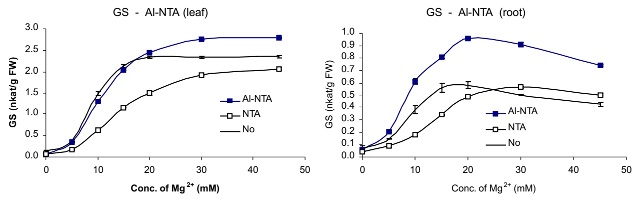

Figure 3. A synthetic complex Al(III)NTA (nitrilo-triacetic acid) is a member the activator group. Highly increases the GS_1 activity, even in the native gels. The ligand itself is inactive, but consumes free Mg^{2+}.

The enhanced GS activity in Al-stressed plants can be considered as a beneficial sideeffect, which can help to reassimilate the stress-born ammonium, preventing loss of nitrogen and ammonium toxicity.

Acknowledgments

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