Environmental Management Science Program

Project ID Number 55118

Plant Rhizosphere Effects on Metal Mobilization and Transport

Teresa W.-M. Fan
University of California at Davis
Hoagland Hall
One Shields Ave.
Davis, California 95616-8627
Phone: 530-752-1450
E-mail: twfan@ucdavis.edu

June 1, 1998

Plant Rhizosphere Effects on Metal Mobilization and Transport

Teresa W.-M. Fan, University of California at Davis

Research Objective

Introduction and Objectives

Information on the mechanism of how plants mobilize, uptake, and metabolize metal ions is very limited. Especially deficient is the understanding of these processes involving pollutant metal ions and interactions among these ions. Based on our current knowledge regarding nutrient ions, it is clear that elucidation of rhizospheric processes such as exudation of organic ligands by plant roots and plant metabolism/adaptation involving these biogenic chelators is critically important. A mechanistic insight into these processes will advance knowledge in microbe-plant host interactions and how metal ions are mobilized, immobilized, and sequestered by these interactions. This, in turn, is essential to applications such as phytobioremediation and microbioremediation of metal ion pollution.

Root exudation also serves many other important rhizosphere functions including energy supply for microbial degradation of organic pollutants, structuring of microbial community, and the formation of soil humic materials which are considered to be a major sink for both organic and inorganic pollutants. How root exudates function is critically dependent on the chemical nature of exudate components. Therefore, a comprehensive characterization of all major exudate components, regardless of their chemical class, should facilitate the development and implementation of bioremediation for both organic and inorganic pollutants.

Therefore, the objectives of this project are:

- (1) To obtain a comprehensive composition of major organic components in plant root exudates as a function of different metal ions;
- (2) To examine plant metabolic response(s) to these metal ion treatments, with emphasis on biosynthetic pathways of organic ligands; and
- (3) To investigate the effect(s) of soil microbial (e.g. mycorrhizae) association on (1) and (2).

Research Progress and Implications

In the 1st year of the project, we utilized multi-nuclear, two-dimensional NMR and GC-MS techniques to determine all of the major components in barley root exudates under Fe sufficient and deficient conditions. Both structural and quantitative information was obtained directly from crude root exudates without sample fractionation, which enables detection of unknown and unexpected compounds. This greatly facilitates chemical characterization of root exudates and ensures that no major components escape detection.

We found that a number of organic ligands were excreted by barley roots including the phytosiderophore mugineic acids (2 α -deoxymugineic acid, mugineic acid, and 3-epi-hydroxymugineic acid) and a number of amino and organic acids. The amount of mugineic acids excreted was correlated positively with the extent of Fe deficiency, with 3-epi-hydroxymugineic acid being the most prominent component. The increase in mugineic acid excretion was accompanied by an elevation in the tissue content of Cu, Zn, and Mn. The total Fe chelating capacity was also measured using the ferrozine assay and compared with the production of the mugineic acids. We were surprised to find that the mugineic acids may account for only part of the Fe chelating capacity, especially under mild and moderately Fe deficient conditions. Lactate, alanine, g-aminobutyrate (GAB), malate, and glycinebetaine (GB) collectively may contribute to a significant fraction of the Fe chelating capacity.

In light of the known stimulatory effect of alanine and citrate on metal availability to algae (Campbell, 1995), the function of these low molecular weight metabolites as vehicles for Fe or metal uptake in general require further investigation. This work was published in *Analytical Biochemistry* 251, 57-68 (1997).

We then proceeded to apply the above approach to investigate the interaction of elevated cadmium (Cd) with Fe deficiency in gramineous plants. We have completed one each series of cadmium (Cd) treatments of barley, wheat, and rice seedlings under Fe sufficient and deficient conditions. The purpose of these experiments was to examine the role of mugineic acids in Cd uptake and the effect of Cd on the chemistry of root exudates. We found that the chemical composition of root exudates differed substantially among these three plants. The wheat root exudates were dominated by 2i-deoxymugineic acid and a polyol component, the barley root exudates were abundant in 3-epi-hydroxymugineic acid and several amino and organic acids with the polyol a minor component, while the major components of rice root exudates included lactate, acetate, Gly, and glyceraldehyde-3-phosphate.

In the cases of barley and wheat, the excretion of mugineic acids was stimulated by Fe deficiency but suppressed by the Cd treatments, particularly under Fe deficient conditions. For barley, the increase in tissue Cd content was positively correlated with the amino acids (Ala, Gly, GAB, Ser, Thr, Asp, Glu) and glycinebetaine. In comparison, the excretion of the polyol component by wheat roots was greatly enhanced by Cd uptake, and that on a per gram basis, wheat roots and shoots sequestered much more Cd than those of barley. These results indicate that the phytosiderophore mugineic acids may not be important in Cd uptake into gramineous plants and that the polyol component may play a significant role in Cd mobilization and sequestration. We presented these findings at the annual meeting of the Society for Environmental Toxicology and Chemistry-Europe at Bordeaux, France, and two manuscripts are being prepared.

As for rice, detailed analysis of exudate and tissue components is still ongoing. A preliminary analysis indicated that the excretion of glyceraldehyde-3-phosphate may be enhanced by the Cd treatments.

We have also developed methods to probe the dynamic relationships between metal ions, siderophores, and humic substances. Using pyrolysis-GCMS and multidimensional NMR, we found that the bacterial siderophore, desferrioxamine, is capable of extensive association with humic materials. This is significant because all current models and concepts assume exchange of metal ions between competing, but otherwise non-interacting, ligand sites. This work shows that the two interacting ligands significantly reduces the formation of the siderophore-Cd ion complex. This work is being published in *The Analyst*, 1998, in press.

In addition to exudate measurement and experiments, we are developing a convenient analysis of sulfhydryl-rich chelator components in Cd-treated plant tissues. This method is based on the derivatization of sulfhydryl (SH) groups in peptides and proteins by bromobimane, followed by separation and quantification using gel electrophoresis. A preliminary analysis of Cd-treated rice tissues indicated that the production of several small SH-rich peptides (most likely phytochelatins) were greatly enhanced by the Cd treatments.

In summary, it is clear that the composition of root exudates varies substantially among plant species and that different exudate components may be involved in Cd uptake into plant tissues. If understood, the relationship among plant species, exudate chemistry, and metal ion mobility may be utilized to engineer the mobilization or immobilization of a spectrum of metal ions in contaminated soils.

Planned Activities

In the next four months, we are conducting ^{15}N and ^{13}C tracer studies on wheat seedlings in solution cultures to investigate the biosynthesis of excreted ligands such as the polyol component and intracellular ligands as a function of Cd treatments. Such studies should help reveal the metabolic flux and pathways for these ligands, which should facilitate the understanding of the molecular genetics involving metal mobilization and sequestration.

In the next year, we also plan to conduct a similar series of experiments with Al treatments to elucidate the organic ligands involved in Al mobilization/immobilization and tolerance.

To more rapidly gain an understanding of how exudate chemistry effects pollutant ion uptake and how this process interacts with the uptake of other plant mineral ions, we are implementing an energy dispersive X-ray fluorescence (ED-XRF)-based method for convenient quantification of multi-elements ranging from ^{23}Na to lanthanides. The method is non-destructive and applicable to a wide range of sample matrices (e.g. plant powders, soils, aqueous solution) with no sample digestion required. The XRF instrument has been installed recently and a preliminary survey indicates that all macro and micro plant nutrient elements can be simultaneously acquired along with pollutant elements including Al, Cd, Pb, Ag, Sr, As, Se, Ba, and Sn present at ppm level in plant tissues.

Furthermore, we are developing a pyrolysis-GC/MS based microanalysis for exudate ligands that are involved in Fe and Cd mobilization. Our preliminary results indicate that the hydroxamate type siderophores such as desferrioxamine can be analyzed directly in soil matrix. Such direct soil analysis of biogenic ligands and XRF analysis of multi-elements in soils and plant tissues will be employed in the next year to investigate the interaction between exudate chemistry and metal ion sequestration in soil-grown plant systems.