

Annual Report for DOE grant # DE-FGO7-976ER20255 "Plant Rhizosphere Effects on Metal Mobilization and Transport", PI's: Teresa W.-M. Fan, Richard M. Higashi, and David E. Crowley
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During the funding period of '96-'97, we explored the utility of multi-nuclear, two-dimensional nuclear magnetic resonance (NMR) spectroscopy and gas chromatography-mass spectrometry (GC-MS) for a comprehensive analysis of barley root exudates collected under Fe sufficient and deficient conditions. As both structural and quantitative information was obtained directly from crude root exudates using this approach, no tedious sample fractionation was necessary, which will greatly facilitate future chemical characterization of root exudates in general.

We found that the phytosiderophore mugineic acids (including 2'-deoxymugineic acid, mugineic acid, and 3-epi-hydroxymugineic acid) were readily identified and quantified in crude exudate samples along with 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 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 a part of the Fe chelating capacity, especially under mild and moderately Fe deficient conditions. Lactate, alanine, γ -aminobutyrate, malate, and glycinebetaine 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 warrant further investigation. This work is now published in *Analytical Biochemistry* **251**, 57-68 (1997) (see enclosed reprint).

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 and wheat 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 wheat root exudates was very different from that of barley root exudates. Namely, the wheat root exudates were dominated by 2'-deoxymugineic acid and a Tris-like component whereas the barley root exudates were again abundant in 3-epi-hydroxymugineic acid and several amino and organic acids plus the Tris-like component. In both cases, the levels of mugineic acids were stimulated by Fe deficiency but substantially suppressed by the Cd treatment, particularly under Fe deficient conditions. The increase in mugineic acid production was accompanied by an elevation in the tissue content of Cu, Zn, and Mn. In contrast, the level of the Tris-like component in wheat root exudates was significantly enhanced by the Cd treatment under Fe sufficient conditions. This preliminary analysis indicates that the phytosiderophore mugineic acids may not be important in the Cd uptake into gramineous plants and that the Tris-like compound may play a more significant role in this respect. This is consistent with the lack of effect of Fe deficiency on tissue content of Cd in both wheat and barley seedlings. We will complete the data analysis of these experiments shortly and prepare a manuscript based on these findings.

Our next effort will be focused on identifying the Tris-like component via purification by high pressure liquid chromatography (HPLC), followed by structure determination using NMR, electrospray mass spectrometry (ES-MS), and Fourier transform infrared spectroscopy (FT-IR).

Another related area of research that we are conducting involves the interactions among metal ions, siderophores, and soil humates. Such interactions were unveiled in part by our recent

investigation and should have a major impact on the transport, mobility, and bioavailability of metal ions in the rhizosphere. Soil humates represent a major “immobile” ligand pool for both inorganic and organic contaminants in the rhizosphere. However, a molecular-level understanding of this role of soil humates is currently lacking, in part due to the difficulty in characterizing humate structures using conventional approaches. From a preliminary survey, we found that a combination of pyrolysis GC-MS, multinuclear 2-D NMR, and FT-IR techniques provided a powerful approach for chemical characterization of humates. We also found that this approach is valuable in characterizing molecular interactions of humates with metal ions and siderophores such as desferrioxamine B (one of the most powerful Fe chelators). For example, 2-D NMR characterization of a rice soil humate with added desferrioxamine B revealed that these two ligands formed an unexpected complex and that the interactions in the complex were largely mediated through the methylene protons of desferrioxamine B and phenolic/sugar-like functional groups of the humate.