

Report for 2004SD19B: Evaluating Glomalin and Its Role in the Sorption of Organic Contaminants

- Other Publications:

- Mercer, E.J., F.V. Schindler, and J. A. Rice. 2005. Solid-state ¹³C NMR Evaluation of Glomalin Extracted From Soil, 226th National Mtg., Am. Chem. Soc., Geochem. Div., Mar. 2005, San Diego, CA, abstracts.
- Mercer, E.J., F.V. Schindler, and J. A. Rice. 2004. Elemental and Structural Assessment of Glomalin. Sigma Xi Annual Meeting and Student Research Conference. Montréal, Quebec, Canada. Nov. 11-14, 2004.
- Schindler, F.V., E. J. Mercer, and J. A. Rice. 2005. Evaluating the Elemental and Structural Character of Glomalin Extracted From Soil. In review

Report Follows

Annual Progress Report

State Water Resources Institute Program (SWRIP)
March 2004 to February 2005

PART I.

Title: Evaluating Glomalin and Its Role in the Sorption of Organic Contaminants

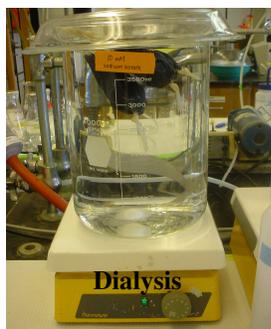
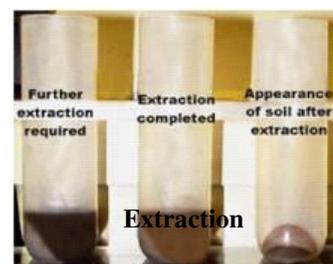
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The following report discusses the results and progression of the research project titled "Evaluating Glomalin and Its Role in the Sorption of Organic Contaminants" during the funding period of March 2004 through February 2005. This project was designed and proposed as a three year project, but received only one year of funding. The information gathered to date provides valuable information regarding glomalin's chemical and structural character, which may have profound implications regarding glomalin's role in contaminant sorption and soil nutrient availability. The objective of this study was to analyze the elemental and structural character of glomalin extracted from whole soils through a series of chemical assays and solid-state NMR and infrared spectroscopic techniques.

Methodology:

Soils. The mineral soil samples used in this study were collected in eastern South Dakota, USA and are described by Malo (1994). They are referred to as the Poinsett silt loam (fine-silty, mixed, superactive, frigid Calcic Hapludolls), and the Hetland (fine, smectitic, frigid, Pachic Vertic Argiudolls). The Pahokee peat, an International Humic Substances Society (IHSS) reference material (Cat. No. BS103P), was used in this study and contained 45% C, 15% ash, 4.7% H, and 3.1% N (IHSS, 2005). All mineral soils were collected as random grabs from the top 0-15 cm of soil after the initial plant litter had been removed (Kohl, 1999). Mineral soil samples were air dried, crushed, and sieved to pass a 2mm mesh.

Glomalin extraction and purification. Glomalin was extracted using the total protein extraction procedure described by Wright et al. (1996). Eight mL of a 50mM sodium citrate solution was placed into a centrifuge tube containing one gram of whole soil. Samples were autoclaved for 60 minutes at 121°C followed by centrifugation at 3000 to 5000 xg for fifteen minutes to pellet the soil particles. The supernatant was decanted and stored at 4°C until purified. Repeated extractions were performed to effect complete glomalin extraction as evidenced by a transparent supernatant (Wright et al., 1996).



To assess the effect of purification method on the chemical and structural characteristics of glomalin, extracted glomalin samples were subjected to either the trichloroacetic acid (TCA) (i.e., Het-TCA, Poin-TCA or Peat-TCA) or hydrochloric acid (i.e., Het-HCl, Poin-HCl or Peat-HCl) precipitation procedures (Wright, 2004). The HCl method is recommended if the carbon (C) and nitrogen (N) concentrations are measured, since TCA may adhere to glomalin during the precipitation process and produce inaccurate C results (Wright et al., 1996). Glomalin extracts were added to centrifuge tubes in a 1:1 ratio with ice cold 20% TCA and incubated for 1 hr. Samples were centrifuged, the supernatant decanted, and the solid

material reconstituted with 1 mL of 100 mM sodium borate solution. This was transferred to hydrated dialysis tubing (3500 Daltons). The tubing was placed in 10 mM borate solution (pH =8) and dialyzed at 8 hr intervals under constant stirring. The dialysis solution was changed at least 3 times to ensure proper purification. The purified dialyzate was centrifuged at 10,000 rpm for 15 min to remove any extraneous particles. The supernatant was transferred to freeze drying flasks, immediately frozen in liquid N₂, and lyophilized. Extracted glomalin samples were also purified and reconstituted similarly except 1N HCl and 0.1 M NaOH were used for precipitation and reconstitution, respectively (Wright, 2004). All reagents used in extraction and purification were purchased from Aldrich Chemical Co. Inc. at 99+% purity.

Protein Assay. The protein content of glomalin extracted from whole mineral and organic soils was quantified using the standard Bradford assay (Bradford, 1976). One hundred mg Coomassie Brilliant Blue G-250 (Sigma-Adrich Co.) was dissolved in 50 mL 95% ethanol and 100 mL of 85% phosphoric acid was added. The resulting solution was diluted to a final volume of 1 L, filtered thorough a Whatman #1 paper, and used as the color reagent for protein quantitation. Standard solutions of reagent grade Bovine Serum Albumin (Equitech-Bio, Inc., Kerrville, TX) were prepared containing 20 to 200 μ g protein. Color reagent was added to both standard and sample unknowns in a 50:1 (v:v) reagent to sample ratio, vortexed, and allowed to incubate for 10 min before absorbance measure at 595 nm. The protein in unknown samples was determined by fitting a least squares regression curve of the quantity of standard protein vs. absorbance. All standard and sample unknowns possessed the same solution matrix.

Solid-state ¹³C NMR. Glomalin was characterized by quantitative solid-state ¹³C NMR using the technique described by Mao et al. (2000). Glomalin samples were placed in a 4 mm o.d. zirconia rotor equipped with Kel-F endcaps and characterized on a Bruker AVANCE 300 (7.4T) widebore spectrometer. All spectra were acquired at 75 MHz using direct-polarization magic angel spinning (DPMAS) combined with a spin-lattice relaxation correction (T₁^C) and total sideband suppression (CP-TOSS) (Mao et al., 2000). T₁^C values used for DPMAS ranged from 3 to 6 sec. High-power pulse



lengths and power levels were optimized with respect to an external reference consisting of a mixture of L-leucine-1-¹³C, glycine-2-¹³C, and L-alanine-3-¹³C in a 1:1:1 ratio. During spectrum acquisition, rotor spin rate and the number of scans were held constant at 13 kHz and 20 000, respectively. The ¹³C NMR spectra were integrated according to the following chemical shift regions: 0-50 ppm = aliphatic carbon, 50-108 ppm = carbohydrate carbon, 108-160 ppm = aromatic carbon, and 160- 200 ppm = carboxyl carbon, and 200-220 ppm = carbonyl carbon (Mao et al., 2000; Wilson, 1987).

Chemical Analyses. Glomalin and BSA samples were sent to Huffman Laboratories, Inc. in Golden, Co. for C, H, N, O, Fe, Na, P, and ash content determinations. Iron, P, and Na were performed after mixed acid decomposition of the samples ending with complete oxidation of organic material by refluxing with perchloric acid. The diluted digestion solutions were analyzed by inductively coupled atomic emission spectroscopy (ICP-AES) following EPA method 200.7 protocol and using a Perkin-Elmer Optima 3000 analyzer. Carbon and H were determined using a custom built analyzer which uses coulometric detection. Ash content was determined by high temperature combustion of the sample until a constant weight was obtained. Moisture content of samples was determined by Karl Fisher titration. All elemental analyses presented in Table 1 are reported on a water-free basis. All non-ash elements reported on a water and ash-free basis.

Principal Findings and Significance:

Selected characteristics of whole soil, glomalin extracted material, and bovine serum albumin (BSA) protein standard are presented in Table 1. The Bradford assay ranged from 54 to 114 mg protein g⁻¹ of extracted material (Table 1), which corresponds to 3 to 68 mg of Bradford sensitive protein g⁻¹ of soil. This range is within that reported for mineral and organic soils (Lovelock et al., 2004; Wright, 2002). On average, glomalin accounted for 25% and 52% of the total C in the mineral soils (Hetland and Poinsett) and organic soil, respectively. This is consistent with the literature for a typical mineral soil (Wright, 2002). Little difference in the elemental composition of glomalin extracted from the Hetland and Poinsett soils was observed, however, marked increases in C and N and decreases in ash percentages were evident with the peat soil. The glomalin extracted from the mineral soils showed higher ash percentages compared to that extracted from peat because of the latter's higher total organic C content. Also, the high Na content among the whole soils was presumably the result of an inadequate purification process. That is, despite careful dialysis of the concentrated protein, Na residue persisted indicating a need to provide greater assurance of protein purity in future extractions. To date, no information regarding glomalin's ash content has been reported in the literature.

It has been reported that glomalin is comprised of N-linked oligosaccharides (Wright and Upadhyaya, 1998; Wright et al., 1998). The presence of N-linked oligosaccharides on arbuscular mycorrhizal (AM) fungus hyphal protein was used to support a proposal that glomalin is a glycoprotein (Wright et al. 1998). If glomalin is a glycoprotein, it would contain significant amounts of mannose units linked to N-acetylglucosamine groups (Kyte, 1995). The NMR spectra of glomalin show very little carbohydrate carbon (Fig. 1). Even BSA, which is not considered a glycoprotein, contains more carbohydrate than the glomalin extractions (Fig. 1 and Table 2). Given glycoproteins high mannose composition, one would have expected that glomalin's ¹³C NMR spectra show a significant carbohydrate fingerprint (Fig. 1). Literature suggests that glomalin contains approximately 60% carbohydrate (Wright and Upadhyaya, 1998) which is contrary to our findings (Table 2 and Fig. 1).

Another interesting observation is how similar glomalin is to humic substances despite suggestion that glomalin's structure differs from that of humic acid (Wright, 2002). The solid-state ¹³C NMR spectrum of the IHSS Pahokee Peat humic acid (PHA) fraction (IHSS, 2005) bears a carbon type distribution similar to that of the glomalin samples evaluated in this study. The PHA contains high aromatic (47%) and carboxyl (20%) carbon types and relatively low aliphatic and carbohydrate carbon (IHSS, 2005). Furthermore, the molar H:C and C:N ratios of the PHA are 0.81 and 17.8, respectively, which are similar to the glomalin ratios reported here (Table 1). Mao et al. (2000) also showed how certain humic acids contain very little aliphatic (12%) and carbohydrate (14%), but large amounts of aromatic and carboxyl carbon (i.e., 48% and 26%, respectively). Mao et al. (2000) discussed how this functional group arrangement could be expected with older humic acids given the long exposure to microorganism attack and the more rapid degradation of the easily decomposing compounds such as proteins, carbohydrates, and phenolic groups. Additional ¹³C NMR experiments need to be performed on the humic acid fractions of the Poinsett and Hetland soils to lend additional support to the humic acid and glomalin structural similarities.

Table 1. Characteristics of whole soil, glomalin extracted material, and bovine serum albumin (BSA) standard. †

Soil Type	Total Organic Carbon††	Mass of Extract¶	Protein Content#	Carbon	Hydrogen	Nitrogen	Oxygen-Merz	Ash	Iron	Phosphorus	Sodium	H:C	C:N
	%	mg g ⁻¹	mg g ⁻¹	—————			%						
Poinsett ‡	3.5	41	64.3	45.3	5.90	3.97	44.8	57.19	2.35	0.0998	7.22	1.6	13.3
Poinsett §	-----	60	53.8	41.4	6.31	3.37	48.9	65.96	1.68	0.0893	10.60	1.8	12.2
Hetland ‡	2.8	50	63.1	48.9	4.89	3.67	42.5	64.04	2.48	0.0726	9.11	1.2	15.6
Hetland §	-----	58	45.1	44.3	5.69	3.60	46.4	74.73	1.57	0.0495	12.43	1.5	14.4
Pahoee Peat ‡	45.1	605	111.8	58.6	4.11	4.14	33.2	31.56	0.536	0.0330	9.05	0.85	14.5
Pahoee Peat §	-----	488	113.7	56.5	4.25	4.04	35.2	17.51	0.488	0.0424	6.30	0.90	16.3
BSA	nd	nd	nd	53.9	7.08	15.5	23.5	1.34	<0.001	0.0148	0.596	1.6	48.6

† All elemental analyses reported on a water-free basis. All non-ash elements (i.e., C, H, N, and O) also reported on an ash-free basis.

‡ 1.0 M HCl precipitation; 0.1 M NaOH reconstitution

§ 20% TCA precipitation; 100 mM sodium borate reconstitution

†† Percent organic C of whole soil

¶ milligrams of extracted material per gram of soil

milligrams of Bradford sensitive protein per gram of extracted material

nd not determined

Table 2. Carbon type distribution of glomalin extracted from whole samples.

Glomalin Type	Aliphatic (0-50 ppm)	Carbohydrate (50-108 ppm)	Aromatic (108-160 ppm)	Carboxyl (160-200 ppm)	Carbonyl (200-220 ppm)
————— % —————					
Poinsett †	9.2	4.7	44.3	30.2	1.6
Poinsett ‡	10.6	9.9	50.8	26.7	2.7
Hetland †	5.3	16.0	49.2	27.7	1.8
Hetland ‡	4.4	7.1	42.1	24.2	2.2
Pahoee Peat †	3.9	11.9	49.1	30.4	4.7
Pahoee Peat ‡	6.1	3.8	41.0	26.6	2.4
BSA	53.5	14.9	11.9	18.9	0.8

† 1.0 M HCl precipitation; 0.1 M NaOH reconstitution

‡ 20% TCA precipitation; 100 mM sodium borate reconstitution

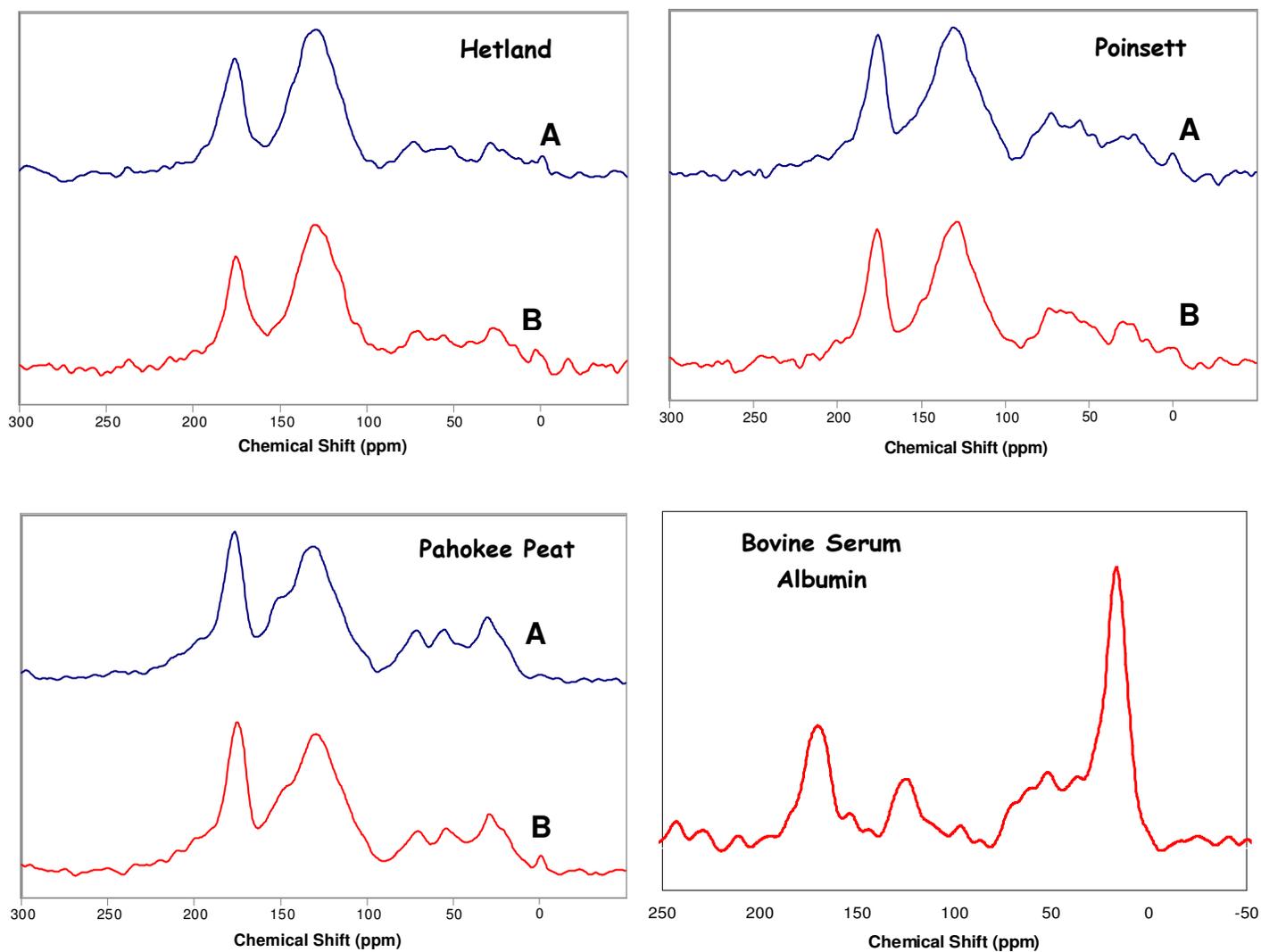


Figure 1. ^{13}C DPMAS NMR spectra of glomalin extracted from Hetland and Poinsett soils, Pahokee peat and bovine serum albumin . HCl precipitation/NaOH reconstitution (A) and TCA precipitation/sodium borate reconstitution (B).

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PART II:

Information Transfer Program: The first year's results have been presented at a national and international meeting and a manuscript has been prepared and is currently in review. Publication of the manuscript in either the Environmental Science and Technology or Soil Science Society of America Journal is imminent. Furthermore, the results of this study, which seem to refute current understanding of glomalin's structural character, have prompted interest and interdepartmental discussions among other researchers at SDSU.

Student Support: This project made it possible for an undergraduate student from St. Olaf College in Northfield, MN, Ms. Erin Mercer, to participate in the National Science Foundation's (NSF) Research Experiences for Undergraduates (REU) program at SDSU. Ms. Mercer was able to present her work at the Sigma Xi Annual Meeting and Student Research Conference in Montréal, Quebec and at the National Meetings of the American Chemical Society in San Diego, California.