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Identification of inositol hexakisphosphate binding sites in soils by selective extraction and solution ^{31}P NMR spectroscopy

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ABSTRACT

Inositol hexakisphosphate (IP_6) can constitute the majority of the organic phosphorus in soil. Soil IP_6 accumulates through a number of mechanisms, including sorption to metal hydroxides and clays, association with organic matter, and precipitation with cations and on surfaces of metal oxides. However, the relative contributions of these processes remain unknown. We quantified IP_6 stereoisomers by NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy in a series of contrasting soils from natural and agricultural ecosystems, and then used selective extractions to identify associations between IP_6 and soil components. Oxalic acid, which extracts amorphous and organically complexed iron and aluminum oxides, extracted the majority of the IP_6 from temperate grassland and forest soils, but not from strongly weathered tropical rice soils. In contrast, removal of mineral material by pretreatment with hydrofluoric acid completely removed IP_6 from temperate forest soils, but not from temperate grasslands or tropical rice soils. We conclude that the relative importance of IP_6 stabilization on organic and mineral components varies markedly among soils, and that oxalate extraction provides a selective procedure for the quantification of IP_6 associated with amorphous metal oxides and clays.

1. Introduction

Inositol hexakisphosphate (IP_6) constitutes the majority of the organic phosphorus (P) in many soils (Turner et al., 2002). The greatest concentrations appear to occur in grassland soils, but IP_6 has also been detected in crop soils, forest soils, and rice paddies (Turner, 2007). Four stereoisomers of IP_6 have been identified in soils; the most abundant is the *myo* isomer, with smaller amounts of the *scyllo*, *neo* and D-*chiro* isomers (Cosgrove, 1962; Cosgrove and Tate, 1963; Turner, 2007; Turner et al., 2012). *myo*-Inositol hexakisphosphate is the main P compound in seeds and is also present in manure from monogastric animals, which are not able to digest *myo*-IP_6 (Cosgrove, 1980; Leytem and Maguire, 2007). The other three stereoisomers do not occur in plant tissue, so are presumably synthesized by soil microbes, perhaps by epimerization of the *myo* isomer (Turner, 2007).

Inositol hexakisphosphate accumulates in soils through interactions with mineral and organic soil components. This can occur by adsorption to aluminum (Al) and iron (Fe) hydroxides/oxides, clays, or calcite, association with organic matter, or precipitation with cations as phytate (salts of *myo*-IP_6) (Celi and Barberis, 2007; Karathanasis and Shumaker, 2009). Adsorption may occur through a ligand exchange mechanism between the phosphate groups and surface reactive OH^- or H_2O groups on the adsorbents (Celi and Barberis, 2007; Ognalaga et al., 1994), although a recent study indicates that the adsorption to goethite occurs as an outer sphere complexation in which hydrogen bonds between the surface of goethite and IP_6 are formed (Johnson et al., 2012). Furthermore, IP_6 may rapidly form surface precipitates of Al–IP_6 complexes on the surface of Al oxides after a brief initial adsorption phase (Yan et al., 2014a). The adsorption capacity of metal oxides for IP_6 increases as soil pH decreases (Celi et al., 2001), which renders associations with calcite and organic matter less important (Celi and Barberis, 2007). Association between IP_6 and soil organic matter might occur via physical or chemical incorporation within organic matter structures, or through adsorption to organic matter via metal bridges (Celi and Barberis, 2007), although only the latter mechanism has been demonstrated experimentally (Leytem et al., 2002). In acidic soils, associations with amorphous Al and Fe hydroxides are believed to be the most important mechanism of IP_6 stabilization (Celi and Barberis, 2007). This is supported by correlations between amorphous metals and IP_6 across a wide variety of soils (Anderson et al., 1974; McKercher and Anderson, 1968; Turner et al., 2007, 2003; Vincent et al., 2012). However, the relative contribution of these stabilization processes remains poorly understood, in part because most recent studies have extracted IP_6 from soils by a single-step NaOH–EDTA procedure, which is assumed to extract mineral associated IP_6 as well as IP_6 associated with the organic soil matrix (Turner et al., 2005a).

Recently, a procedure using dilute hydrofluoric acid (HF) pretreatment followed by NaOH–EDTA extraction has been used to identify P associated with the organic soil matrix (Dougherty et al., 2007;

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Hamdan et al., 2012). Hydrofluoric acid dissolves the mineral matrix but leaves organic matter largely intact (Dougherty et al., 2007). It will therefore remove all IP_6 associated with the mineral matrix, leaving IP_6 associated with organic matter. This can be used to determine whether IP_6 is associated with mineral or organic material, but cannot indicate whether IP_6 is associated with amorphous or crystalline metal hydroxides. However, extraction in acidic ammonium oxalate is routinely used to extract amorphous Al and Fe hydroxides, as well as associated P (Gleyzes et al., 2002). The oxalate extract acts via a ligand exchange with surface OH^- groups and forms a complex (e.g. Fe(III)–$C_2O_4^{2-}$) that polarizes and weakens the metal–O bonds between metal atoms and the surface of the metal complex, leading to non-reductive dissolution (Gleyzes et al., 2002; Stanjek and Weidler, 1992; Zinder et al., 1986). Thus, the specificity for amorphous metal complexes is due to their relatively high specific surface area (concentration of OH^- per area) leading to a high solubility compared to crystalline forms such as goethite (Karim, 1984; Schwertmann, 1973; Theng et al., 1982). The apparent strong association between amorphous metals and IP_6 in soils suggests that oxalate extraction offers potential as a selective extractant for IP_6 associated with amorphous metals.

Given the importance of soil organic P for plant nutrition in both natural and agricultural ecosystems (Richardson et al., 2005), there is an urgent need to develop procedures that provide accurate information on the nature and stabilization of organic P compounds in soils. We used HF pretreatment and oxalate extraction in combination with solution ^{31}P NMR spectroscopy to assess the association of IP_6 stereoisomers with organic matter and metal oxides. Our aim was to separate IP_6 into: (i) IP_6 bound to amorphous Al and Fe, and (ii) IP_6 associated with organic matter. In theory, this would allow calculation of a third group of IP_6 bound to more crystalline metal oxides. The procedure was tested on a series of seven soils known to contain inositol phosphates from three different ecosystems: temperate grasslands, tropical rice fields and lowland temperate rainforest.

2. Methods

2.1. Locations, soil sampling, and preparation

Soil was collected from three ecosystems: tropical rice paddies in Madagascar (Turner, 2006), temperate grasslands in the Falkland Islands (Turner et al., 2012), and temperate rainforest at the Haast chronosequence in New Zealand (Turner et al., 2014). The following labels were used: for the rice paddies (MDG), temperate grassland soils (EAST) and temperate rainforest soils (Dune). The soils had a range of properties, including total P concentrations (Table 1), and were known from previous studies to contain IP_6. Detailed information on the soils is available elsewhere (Turner, 2006; Turner et al., 2012, 2014), although it should be noted that the temperate grassland soils were from slightly different locations from those studied previously (Turner et al., 2012). All samples were surface soils (0–10 cm) and were air dried, screened and sieved (<2 mm) prior to analysis, with storage in sealed plastic bags at ambient laboratory temperature and humidity (22 °C and 55%, respectively).

2.2. NaOH–EDTA extraction

Total IP_6 was extracted by shaking soil (1.00 ± 0.01 g) in 20 mL of a solution containing 0.25 M NaOH and 0.05 M Na_2EDTA (disodium ethylenediaminetetraacetate) for 16 h (Turner et al., 2005a). Extracts were centrifuged at 10,000 *g* for 10 min and the supernatant decanted. Each solution was spiked with 1 mL 50 μg mL^{-1} methylene diphosphonic acid (MDP) as an internal standard, frozen at −40 °C, and lyophilized. We assume that the NaOH–EDTA procedure yields quantitative recovery of organic P and therefore IP_6 from soils, although this remains poorly understood given the lack of a procedure for the direct determination of total soil organic P (Turner et al., 2005a).

2.3. Pretreatment with hydrofluoric acid

To isolate IP_6 associated with soil organic matter, soils were pre-extracted in 10% HF according to the procedure of Hamdan et al. (2012). Briefly, soil (2.0 ± 0.01 g) was extracted four times in 45 mL of 10% HF for 1 h, and then twice for 24 h. The solution was centrifuged at 1790 *g* for 10 min between each step and the supernatant discarded. After the final HF treatment, the soil pellet was rinsed five times in 45 mL distilled water, dried, weighed, and extracted in 30 mL of NaOH–EDTA. The extracts were then frozen, spiked with internal standard, and lyophilized as described above.

2.4. Oxalate extraction

To extract IP_6 associated with amorphous metal oxides, soil (1.00 ± 0.01 g) was extracted in 40 mL of a solution containing 0.2 M ammonium oxalate monohydrate ($(NH_4)_2C_2O_4 \cdot H_2O$)–oxalic acid ($C_2H_2O_4 \cdot 2H_2O$) adjusted to pH 3 (Schwertmann, 1964). The samples were shaken in darkness for 2 h and then centrifuged for 10 min at 2000 *g*. Two milliliters of sample were diluted in 2% HNO_3 and analyzed for Al, Fe, and P by inductively-coupled plasma optical-emission spectroscopy (ICP-OES) (Optima 7300DV, Perkin Elmer, Shelton, CT). The pH of the remaining supernatant was increased to ~8 by addition of NaOH, 20 g of amberlite cation exchange resin (chelex 100 resin; Sigma-Aldrich) was added, and the mixture shaken for 1 h. The supernatant was removed and the resin was washed three times with 10 mL of distilled water. The washings were added to the supernatant and pH was increased to >12 by addition of NaOH. Each solution was spiked with 1 mL of 50 μg P mL^{-1} MDP, frozen at −40 °C, and lyophilized. Samples were kept in darkness throughout the procedure to avoid degradation of the oxalic acid.

Table 1
Locations and soil properties adopted from published papers. Concentrations of Al, Fe, and P (mg kg^{-1}) in the oxalate extracts determined by ICP-OES and P saturation (P_{sat}) = $P_{ox} * 100 / (Al_{ox} + Fe_{ox})$, expressed as molar ratios.

Location code	Location	Total elements			pH	Oxalate extractable			Psat	Topsoil texture	Taxonomic order	Vegetation	Reference
		P	C	N		Al_{ox}	Fe_{ox}	P_{ox}					
		mg P kg^{-1}	(%)	(%)		g Alkg^{-1}	g Fekg^{-1}	mg P kg^{-1}	%				
MDG 8	Madagascar	828	6.4	0.53	5.0	8.34	6.01	385	3.0	Clay	Oxisol	Tropical rice paddy	Turner (2006)
MDG 10	Madagascar	1128	3.3	0.39	5.0	2.31	4.99	189	3.5	Clay	Oxisol	Tropical rice paddy	Turner (2006)
EAST 46	Falkland Islands	1376	16.0	1.10	5.3	5.91	3.59	952	10.9	n.d.[c]	Spodosol	Temperate grassland	Turner et al. (2012)
EAST 48	Falkland Islands	1213	13.8	0.98	5.2	7.15	4.48	809	7.6	n.d.[c]	Spodosol	Temperate grassland	Turner et al. (2012)
EAST 54	Falkland Islands	995	12.5	0.92	5.5	4.18	4.49	657	9.0	n.d.	Spodosol	Temperate grassland	Turner et al. (2012)
Dune 3[a]	New Zealand	229	3.1	0.16	4.2	0.68	2.37	140	6.7	Loamy sand	Entisol	Temperate rainforest	Turner et al. (2014)
Dune 8[b]	New Zealand	155	1.9	0.09	3.9	1.38	3.00	97	3.0	Loamy sand	Entisol	Temperate rainforest	Turner et al. (2014)

[a] Age: 392 years old.
[b] Age: 1826 years old.
[c] n.d. = not detected.