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QUANTIFICATION OF *MYO*-INOSITOL HEXAKISPHOSPHATE IN ALKALINE SOIL EXTRACTS BY SOLUTION ^{31}P NMR SPECTROSCOPY AND SPECTRAL DECONVOLUTION

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Inositol phosphates are the dominant class of organic phosphorus (P) compounds in most soils, but they are poorly understood because they are not easily identified in soil extracts. This study reports a relatively simple technique using solution ^{31}P NMR spectroscopy and spectral deconvolution for the quantification of *myo*-inositol hexakisphosphate (phytic acid), the most abundant soil inositol phosphate, in alkaline soil extracts. An authentic *myo*-inositol hexakisphosphate standard added to a re-dissolved soil extract gave signals at 5.85, 4.92, 4.55, and 4.43 ppm in the ratio 1:2:2:1. Spectral deconvolution quantified these signals accurately (102 ± 4%) in solutions containing a mixture of model P compounds by resolving the envelope of signals in the orthophosphate monoester region. In NaOH-EDTA extracts from a range of lowland permanent pasture soils in England and Wales, concentrations of *myo*-inositol hexakisphosphate determined by spectral deconvolution ranged between 26 and 189 mg P kg^{-1} soil, equivalent to between 11 and 35% of the extracted organic P. Concentrations were positively correlated with oxalate-extractable aluminum and iron but were not correlated with total carbon, total nitrogen, clay, or the microbial biomass. This suggests that *myo*-inositol hexakisphosphate accumulates in soils by mechanisms at least partially independent of those controlling organic matter stabilization and dynamics. Furthermore, *myo*-inositol hexakisphosphate concentrations were positively correlated with plant-available inorganic P and negatively correlated with the carbon-to-organic P ratio, suggesting that biological P availability may, in part, regulate *myo*-inositol hexakisphosphate concentrations in soils, perhaps because organisms capable of degrading this compound are favored in more P-limited environments. Solution ^{31}P NMR spectroscopy and spectral deconvolution offers a relatively simple method of quantifying *myo*-inositol hexakisphosphate in soil extracts. (Soil Science 2003;168:000–000)

Key words: Pasture soil, inositol phosphate, *myo*-inositol hexakisphosphate, phytic acid, solution ^{31}P NMR spectroscopy, spectral deconvolution.

INOSITOL phosphates are the dominant class of organic phosphorus (P) compounds in most soils. These ubiquitous compounds are derived principally from plants as *myo*-inositol hexakisphosphate, which functions as a storage compound in seeds. *myo*-inositol hexakisphosphate, also known as phytic acid, is also the most abundant inositol phosphate in soils, although a number of lower esters (e.g., pentakisphosphate, tetrakisphosphate) and stereoisomers (*scyllo*, D-*chiro*, *neo*) occur in smaller amounts (Turner et al., 2002). Inositol phosphates typically represent less than 50% of the soil organic P (Anderson, 1964;

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Williams and Anderson, 1968), although they may constitute almost all the organic P in some calcareous soils (Jencks et al., 1964).

The abundance of inositol phosphates in most soils means that there is currently much interest in their behavior, availability to plants, and fate in the environment (Celi et al., 1999; Richardson et al., 2001; Turner et al., 2002). However, such information is limited, mainly because the extraction, separation, and detection of inositol phosphates in environmental samples remains a complex and problematic procedure. Inositol phosphates are extracted conventionally from soils in an alkaline solution, which is then cleaned and the phosphates separated by chromatography (e.g., McKercher and Anderson, 1968b). However, such techniques are time consuming and compromised by poor recoveries of inositol phosphates from soils and chromatography columns.

The analysis of soil organic P by chromatography has largely been superceded by solution ^{31}P NMR spectroscopy. This technique obviates many of the problems associated with chromatography by allowing the simultaneous determination of functional P groups without the need for lengthy separation and clean-up procedures (Condron et al., 1997). However, solution ^{31}P NMR spectroscopy remains limited to the specific analysis of inositol phosphates because poor resolution in the orthophosphate monoester region of the spectrum obscures individual signals from these compounds, especially at low field strengths. Nevertheless, a procedure for quantifying inositol phosphates in soil extracts by solution ^{31}P NMR spectroscopy would provide an important tool for investigating these enigmatic compounds.

Here we describe a novel procedure for quantifying *myo*-inositol hexakisphosphate in alkaline soil extracts using solution ^{31}P NMR spectroscopy and spectral deconvolution software. The deconvolution procedure resolves overlapping signals in the NMR spectrum into their component peaks. This requires a degree of operator input, in particular to determine the number and characteristics of the deconvoluted peaks, but it more accurately quantifies signals from individual compounds where these can be identified using authentic standards. Deconvolution has rarely been used to study soils, although ^{13}C CP-MAS spectra of decayed wood, dissolved organic carbon, and fresh cedar foliage (Davis et al., 1994; Wershaw et al., 1996; Preston, 2001), and solid-state ^{31}P spectra of agricultural soils (Lookman et al., 1996; McDowell et al., 2002) have all been investigated using the technique. In this study we demonstrate the application of the deconvolution procedure to the determination of *myo*-inositol hexakisphosphate in a range of permanent lowland pasture soils from England and Wales, and we use this information to explore the factors controlling *myo*-inositol hexakisphosphate accumulation in the soil environment.

MATERIALS AND METHODS

Soil Sampling and Analysis

Twenty nine lowland permanent pasture soils from England and Wales were selected from the National Soil Inventory database (National Soil Resources Institute, Cranfield University, UK) to give a wide range of physical and chemical properties. Mean annual temperature across the sites ranges between 8.5 and 11 °C, while mean annual rainfall ranges between 600 and 850 mm. Soils were taken to 10 cm, coarsely sieved (4 mm), air-dried (30 °C), then re-sieved (2 mm) before extraction. We recently reported the general P composition of these soils determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy (Turner et al., 2003b). Total carbon (C) and nitrogen (N) were determined simultaneously using a Carlo-Erba model NA2000 analyser (Carlo-Erba, Milan, Italy). Total soil P was determined by NaOH fusion (Smith and Bain, 1982). Textural information was obtained by wet sieving followed by analysis using a Micromeritics Sedigraph 5100 with a Micromeritics Mastertech 51 automatic sampler (Micrometrics, Norcross GA). Soil pH was determined in a 1/2.5 soil/deionized water ratio. Microbial C, N, and P concentrations were determined by fumigation-extraction procedures and were previously reported (Turner et al., 2001). Oxalate-extractable aluminum (Al) and iron (Fe) were determined by extraction with ammonium oxalate/oxalic acid (pH 3.0) for 2 h with detection by ICP-AES (Schoumans, 2000). Plant-available P was determined by extraction in 0.5 M $NaHCO_3$ (pH 8.5) for 30 min with detection by molybdate colorimetry (Olsen et al., 1954).

NaOH-EDTA Extraction and Solution ^{31}P NMR Spectroscopy

Phosphorus was extracted by shaking 5 g of soil with 100 mL of a solution containing 0.25 M NaOH and 0.05 M EDTA for 16 h at 20 °C (Cade-Menun and Preston, 1996). The extracts were centrifuged (10,000 × g for 30 min), frozen at −30 °C, and lyophilized over several days. Total P in the extracts was determined by mo-

lybdate colorimetry following acid-persulphate digestion (Rowland and Haygarth, 1997). The extracts were initially diluted (100-fold with deionized water) to prevent interference from EDTA in the colorimetric procedure. All analytical results are means of three replicate analyses with standard errors (not shown) less than ± 5% of the mean value.

Freeze dried NaOH-EDTA extracts (approximately 200 mg) were re-dissolved in 1 mL of 1 *M* NaOH and 0.1 mL D_2O (for signal lock) and transferred to 5-mm-diameter NMR tubes. The addition of NaOH ensures consistent chemical shifts and optimum spectral resolution at a solution pH > 12 (O'Neill et al., 1980). Solution ^{31}P NMR spectra were obtained using a Bruker AMX 600 spectrometer operating at 243 MHz for ^{31}P. We used a 30° pulse width, a total acquisition time of 1.5 s (pulse delay 0.808 s, acquisition time 0.672 s) and broadband proton decoupling. Temperature was regulated at 24 °C, although it is now recommended that temperature should be standardized at 20 °C for solution ^{31}P NMR spectroscopy (Cade-Menun et al., 2002; Turner et al., 2003a). The number of scans collected (4000–40,000) depended on the P concentration of the freeze-dried extract. Chemical shifts were determined relative to 85% orthophosphoric acid after convolution with a Lorentzian width of 10 Hz. The chemical shifts and area of individual signals were determined using the deconvolution process of the Bruker WinNMR program.

Identification of myo-*Inositol Hexakisphosphate by Spectral Deconvolution*

Signals from *myo*-inositol hexakisphosphate were identified by spiking a re-dissolved NaOH-EDTA extract with an authentic *myo*-inositol hexakisphosphate standard (Sigma P 7660; phytic acid, magnesium-potassium salt). In each soil extract, signals at these chemical shifts were identified and the sum of their signal areas (as a % of the total NMR signal area) multiplied by the total P concentration in the extract to give the concentration of *myo*-inositol hexakisphosphate. Total organic P in the NaOH-EDTA extracts was determined by summing the areas under the signals from orthophosphate monoesters (3 to 6 ppm), orthophosphate diesters (−1 to 2 ppm), and phosphonates (19 to 21 ppm) (Turner et al., 2003a).

The accuracy of the deconvolution procedure was tested by analyzing solutions containing mixtures of P compounds that included *myo*-inositol hexakisphosphate. Compounds were dissolved in 1 *M* NaOH and included calcium phosphate (monobasic), 2-aminoethylphosphonic acid, choline phosphate (phosphorylcholine chloride), α-D-glucose-1-phosphate (sodium salt) and *tetra*-sodium pyrophosphate, all purchased from Sigma Chemicals (UK). The recovery of *myo*-inositol hexakisphosphate by spectral deconvolution was determined as a proportion (%) of that in the mixture.

RESULTS

Conformation of myo-Inositol Hexakisphosphate at pH > 12

Recent studies using solution ^{1}H NMR spectroscopy have demonstrated that *myo*-inositol hexakisphosphate has a 1-axial/5-equatorial structure between pH 0.5 and 9.0 and a 5-axial/1-equatorial structure between pH 10.0 and 13.0 (Fig. 1); at pH 9.5 both conformations are in dynamic equilibrium (Barrientos and Murthy, 1996). As solutions for NMR spectroscopy in our experiments were pH > 12, *myo*-inositol hexakisphosphate was in the 5-axial/1-equatorial con-

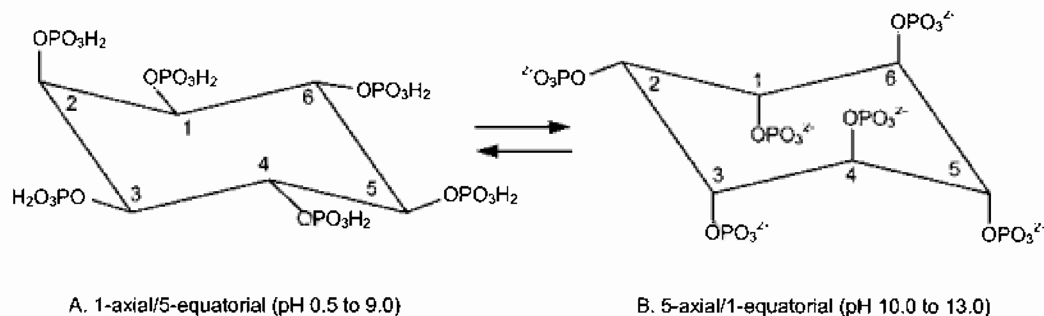

Fig. 1. Structure of *myo*-inositol hexakisphosphate in solution: (a) between pH 0.5 and 9.0, (b) between pH 10.0 and 13.0 (Barrientos and Murthy, 1996).

formation (Fig. 1). In this conformation, only the phosphate in the C-2 position on the inositol ring is equatorial; the remainder are axial. Adoption of this conformation at high pH reduces electrostatic repulsion from dianionic phosphate groups (Barrientos and Murthy, 1996).

Identification of Signals from myo-*Inositol Hexakisphosphate*

The commercial sample of *myo*-inositol hexakisphosphate added to a re-dissolved soil extract gave signals at 5.85, 4.92, 4.55, and 4.43 ppm (Fig. 2). These signals occurred in the ratio 1:2:2:1, corresponding to the positions of the phosphate groups on the inositol ring (Turner et al., 2003a). Thus, the signal at 5.85 ppm corresponded to the equatorial C-2 phosphate, the signal at 4.92 ppm corresponded to the axial C-4 and C-6 phosphates, the signal at 4.55 ppm to the axial C-1 and C-3 phosphates, and, finally, the signal at 4.43 corresponded to the axial C-5 phosphate (Fig. 2).

Determination of *myo*-inositol hexakisphosphate by spectral deconvolution in solutions containing mixtures of model P compounds agreed well with the amounts added (Table 1). The mean recovery of *myo*-inositol hexakisphosphate was 101.8 ± 3.7%.

Soil Properties

Total C concentrations ranged between 28.9 and 80.4 g C kg^{-1} soil, total N concentrations between 2.85 and 8.70 g N kg^{-1} soil, clay contents between 219 and 681 g kg^{-1}, and pH values between 4.4 and 6.8 (Table 2). Total soil P ranged between 376 and 1981 mg P kg^{-1} soil. Concentrations of microbial C (412–3412 mg C kg^{-1} soil), N (57–346 mg N kg^{-1} soil), and P (31–239 mg P kg^{-1} soil) were relatively high in these soils (Turner et al., 2001). Concentrations of oxalate-extractable Al ranged between 0.80 and 3.30 g Al kg^{-1} soil, and oxalate-extractable Fe ranged between 2.92 and 12.58 g Fe kg^{-1} soil (Table 2). Plant-available inorganic P ranged between 9 and 48 mg P kg^{-1} soil.

Concentrations of myo-*Inositol Hexakisphosphate in Soil Extracts*

Soil organic P concentrations determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy ranged from 208 to 895 mg P kg^{-1}, of which between 82 and 95% were orthophosphate monoesters (Table 3). Fig. 3 shows an example of the four signals from *myo*-inositol hexakisphosphate (shaded) in a deconvoluted spectrum of a soil NaOH-EDTA extract. Concentrations of *myo*-inositol hexakisphosphate in the extracts determined by deconvolution of the solution ^{31}P NMR spectra ranged from 26 to 189 mg P kg^{-1} soil (Table 3). These concentrations were equivalent to between 11 and 35% of the NaOH-EDTA extractable organic P, and between 13 and 39% of the total orthophosphate monoesters.

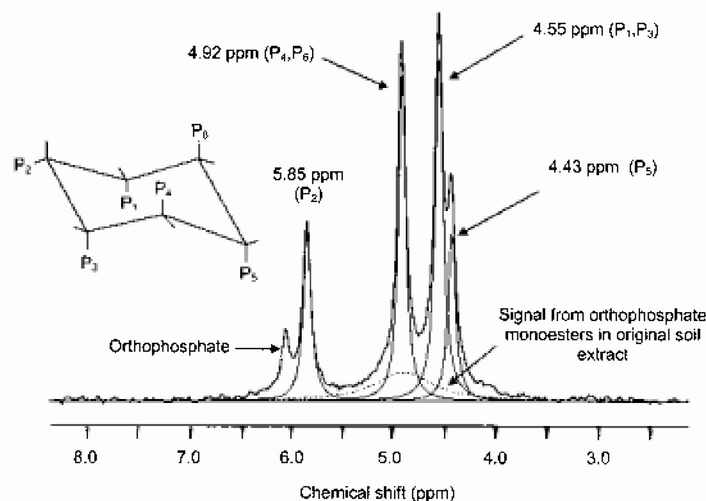

Fig. 2. A deconvoluted solution ^{31}P NMR spectrum of an authentic *myo*-inositol hexakisphosphate standard added as a spike to an NaOH-EDTA soil extract. P = OPO_3^{2-} at pH > 12. The extract was of a Fladbury clay, from Glastonbury, Somerset, and the spectrum of the extract alone is shown in detail in Fig. 3.

TABLE 1

Determination of *myo*-inositol hexakisphosphate by solution ^{31}P NMR spectroscopy and spectral deconvolution in solutions containing mixtures of various model P compounds dissolved in 1 *M* NaOH

Phosphorus compound	Mixture				
	A	B	C	D	E
			% total P		
myo-inositol hexakisphosphate	83.5	37.5	32.1	16.0	35.5
Calcium orthophosphate	16.5				11.8
2-aminoethylphosphonic acid		62.5	48.4	14.8	16.8
Choline phosphate				33.9	5.8
α-D-glucose phosphate				10.8	3.0
Creatine phosphate			19.8	15.1	8.8
Tetra-sodium pyrophosphate				9.4	18.2
myo-inositol hexakisphosphate calculated by deconvolution	87.1	42.8	30.6	14.9	36.5
Recovery of *myo*-inositol hexakisphosphate	*104%*	*114%*	*95%*	*93%*	*103%*

TABLE 2

Physical and chemical properties of lowland permanent pasture soils from England and Wales. The soils are ranked in order of their total C content

Soil series	Location	Textural class†	pH (water)	Clay	Total C	Total N	Oxalate Al	Oxalate Fe
				g kg^{-1} soil				
Brockhurst	Honiton, Devon	Clay	5.0	379	28.9	3.74	1.09	4.67
Dunkeswick	Harrogate, Yorkshire	Clay loam	4.7	346	29.6	3.16	1.19	6.19
Wick	Northallerton, Yorkshire	Sandy clay loam	5.5	219	30.6	3.25	0.85	4.48
Denbigh	Kendal, Cumbria	Clay loam	5.5	273	30.8	3.40	2.44	3.44
Nupend	Kirkham, Lancashire	Clay loam	5.0	240	32.8	3.57	1.13	4.43
Brickfield	Corbridge, Northumberland	Sandy clay	4.8	338	36.9	3.63	1.02	3.66
Dunkeswick	Derby, Derbyshire	Clay	6.1	483	38.9	2.85	1.29	4.67
Brickfield	Haydon Bridge, Northumberland	Sandy clay	4.8	430	39.8	3.40	1.07	4.29
Whimple	Leamington Spa, Warwickshire	Clay loam	5.5	328	40.1	4.05	0.80	3.22
Salop	Whitchurch, Shropshire	Sandy clay	5.1	318	42.3	4.45	1.05	5.15
Whimple	Lower Langford, Somerset	Clay	5.1	338	43.7	4.73	1.00	4.69
Newport	Wem, Shropshire	Sandy clay loam	4.9	250	44.0	4.58	0.82	3.14
Denbigh	Atherstone, Warwickshire	Clay	6.2	401	44.9	4.43	2.06	6.15
Clifton	Chorley, Lancashire	Clay	4.4	457	45.4	4.08	1.46	5.53
Brickfield	Llangefni, Anglesey	Clay loam	4.8	335	46.0	4.83	1.53	5.33
Wick	Haltwhistle, Northumberland	Sandy clay loam	5.9	261	47.2	4.61	1.95	6.30
Brickfield	Dwyran, Anglesey	Clay	5.0	362	47.2	5.04	1.84	6.60
Oxpasture	Cheddar, Somerset	Silty clay	4.8	424	47.5	4.93	2.48	8.94
Whimple	Stafford, Staffordshire	Clay loam	5.1	299	48.0	4.83	0.80	2.92
Hallsworth	Holsworthy, Devon	Clay	4.7	484	48.4	5.37	1.60	9.96
Brickfield	Hexham, Northumberland	Sandy clay	5.0	359	49.6	4.66	3.24	9.01
Moor Gate	Camelford, Cornwall	Clay loam	5.6	313	56.0	5.92	4.95	6.34
Denbigh	Camelford, Cornwell	Clay	4.5	445	58.7	6.35	1.44	7.65
Nordrach	Buxton, Derbyshire	Clay	6.8	541	60.2	5.49	3.30	9.60
Newchurch	Bumham-on-Sea, Somerset	Clay	5.9	547	64.4	6.52	1.35	5.93
Denchworth	Leppington, Yorkshire	Clay	5.8	579	66.3	6.99	1.09	7.09
Worcester	Chew Stoke, Somerset	Clay	6.0	567	67.7	7.57	0.91	2.69
Whimple	Radbourne, Derbyshire	Clay	5.2	629	68.8	7.33	1.51	4.35
Fladbury	Glastonbury, Somerset	Clay	5.0	681	80.4	8.70	2.44	12.88

†Based on topsoil texture.

TABLE 3

Concentrations of total P, organic P, and *myo*-inositol hexakisphosphate in lowland permanent pasture soils from England and Wales

Soil series	Total P mg P kg^{-1} soil	Organic P† mg P kg^{-1} soil	*myo*-inositol hexakisphosphate mg P kg^{-1} soil	*myo*-inositol hexakisphosphate % organic P‡
Brockhurst	1020	418	98	23 (25)
Dunkeswick	519	258	47	18 (21)
Wick	834	267	43	16 (19)
Denbigh	1021	479	141	29 (33)
Nupend	923	350	79	23 (27)
Brickfield	585	314	107	34 (36)
Dunkeswick	821	217	28	13 (16)
Brickfield	376	219	61	28 (30)
Whimple	568	208	26	13 (13)
Salop	821	435	91	21 (23)
Whimple	988	383	88	23 (25)
Newport	962	439	141	32 (36)
Denbigh	1321	424	99	23 (26)
Clifton	571	316	84	27 (31)
Brickfield	1106	545	144	26 (31)
Wick	1312	567	142	30 (34)
Brickfield	854	484	74	15 (18)
Oxpasture	887	418	94	23 (26)
Whimple	833	354	83	24 (26)
Hallsworth	997	416	79	19 (21)
Brickfield	626	275	74	27 (29)
Moor Gate	1004	457	104	23 (26)
Denbigh	1542	895	189	21 (24)
Nordrach	1074	478	167	35 (39)
Newchurch	784	252	35	14 (16)
Denchworth	900	380	64	17 (20)
Worcester	989	397	83	21 (23)
Whimple	1007	488	55	11 (13)
Fladbury	1981	882	163	18 (21)

†Determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy.

‡Values in parentheses are the proportion (%) of orthophosphate monoesters as *myo*-inositol hexakisphosphate.

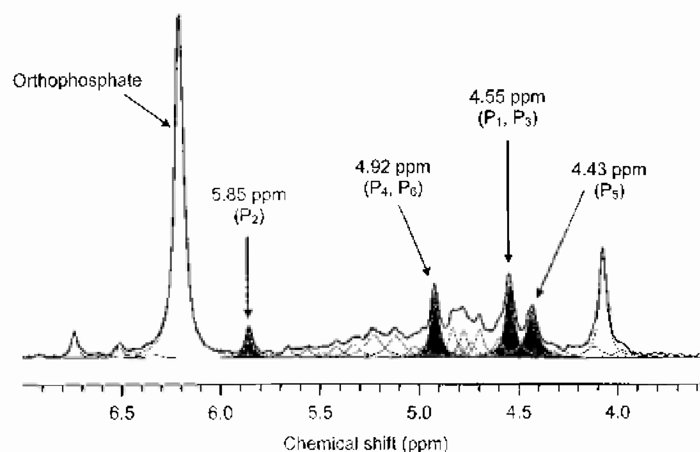

Fig. 3. Identification of signals from *myo*-inositol hexakisphosphate (shaded peaks) in an NaOH-EDTA extract of a lowland permanent pasture soil by solution ^{31}P NMR spectroscopy and spectral deconvolution. P = OPO_3^{2-} at pH > 12. The extract was of a Fladbury clay, from Glastonbury, Somerset (*myo*-inositol hexakisphosphate concentration 163 mg P kg^{-1}).

Relationships Between myo-Inositol Hexakisphosphate Concentrations and Soil Properties

Concentrations of *myo*-inositol hexakisphosphate were correlated positively with total soil P (r = 0.69, P < 0.001), and NaOH-EDTA extractable organic P (r = 0.80, P < 0.001) (Fig. 4), and also with concentrations of inorganic orthophosphate (r = 0.58, P < 0.001), orthophosphate monoesters (r = 0.81, P < 0.001), orthophosphate diesters (r = 0.63, P < 0.001), oxalate-extractable P (r = 0.54, P < 0.01), oxalate-extractable Al + Fe (r = 0.39, P < 0.05), and plant-available inorganic P (r = 0.46, P < 0.05). No statistically significant correlations existed between *myo*-inositol hexakisphosphate and total C, total N, clay, or the microbial biomass, although negative correlations existed between *myo*-inositol hexakisphosphate and the ratios of total C to organic P (r = −0.67, P < 0.001) and total N to organic P (r = −0.47, P < 0.01) (Fig. 4).

DISCUSSION

Solution ^{31}P NMR spectroscopy and spectral deconvolution quantified concentrations of *myo*-inositol hexakisphosphate accurately in model solutions containing a mixture of P compounds and allowed the identification and quantification of this compound in the complex spectra of soil NaOH-EDTA extracts. This technique has considerable advantages over conventional chromatography, which involves lengthy clean-up and separation steps and is susceptible to numerous potential sources of error. The spectral deconvolution technique may not be suitable for all types of extracts because it requires solution ^{31}P NMR spectra that are well resolved in the orthophosphate monoester region. However, where organic P concentrations are low, resulting in poorly resolved spectra, the deconvolution procedure could be improved by using alkaline bromination to pre-treat soil extracts. This technique hydrolyzes all organic P compounds except the inositol phosphates (Irving and Cosgrove, 1981) and will improve resolution in the orthophosphate monoester region considerably.

It is possible that lower esters of *myo*-inositol could give signals at chemical shifts similar to those from *myo*-inositol hexakisphosphate and, therefore, contribute to the signals quantified as this compound by the deconvolution procedure. However, this seems unlikely because lower inositol phosphates constitute a relatively small proportion of the total inositol phosphates in most soils (Omotoso and Wild, 1970; Anderson and Malcolm, 1974) and yield signals with chemical shifts different from *myo*-inositol hexakisphosphate in alkaline solutions. For example, Kemme et al. (1999) demonstrated that a mixture of three *myo*-inositol phosphate esters in a solution of pH 12.6 yielded signals at 5.77, 4.45, 3.96, and 3.78 for *myo*-inositol hexakisphosphate, 5.00, 4.58, 4.34, 3.50, and 3.31 for *myo*-inositol pentakisphosphate, and 4.77 and 3.31 for *myo*-inositol tetrakisphosphate. O'Neill et al. (1980) demonstrated similar differences in acidic solutions, using phytase to generate lower esters of *myo*-inositol.

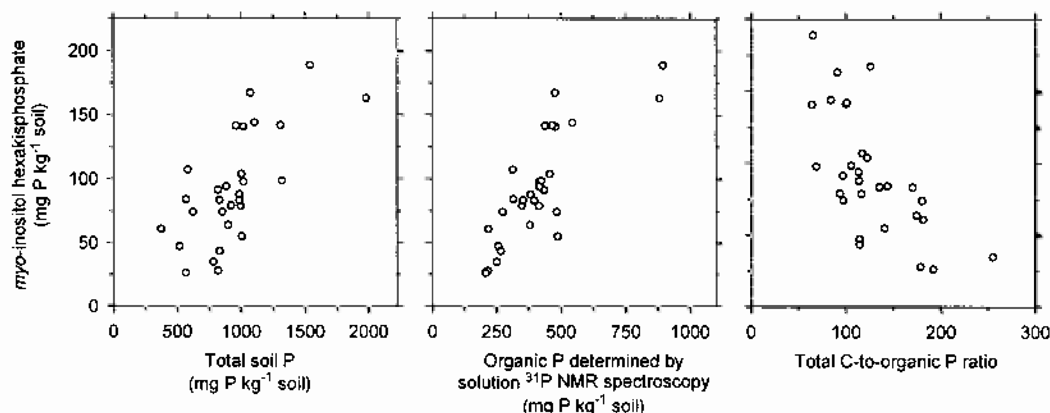

Fig. 4. Relationships between concentrations of *myo*-inositol hexakisphosphate and (a) total soil P, (b) organic P, and (c) the total C-to-organic P ratio, in lowland permanent pasture soils from England and Wales. *myo*-inositol hexakisphosphate was extracted in NaOH-EDTA and quantified by solution ^{31}P NMR spectroscopy and spectral deconvolution. Organic P was calculated by the same procedure as the sum of orthophosphate monoesters, orthophosphate diesters, and phosphonates.

The potential contribution from inositol phosphate stereoisomers other than *myo* is more difficult to assess. Of the eight other possible stereoisomers, only *scyllo*, D-*chiro* and *neo* have been identified in soils, all of which differ from *myo* by the orientation of a single P group (Turner et al., 2002). Proportions vary widely but usually occur in the order of *scyllo* > D-*chiro* > *neo* (McKercher and Anderson, 1968a; Oniani et al., 1973; Irving and Cosgrove, 1982). Little information exists on the solution ^{31}P NMR chemical shifts of these isomeric inositol phosphates, at least in soil extracts, in part because of the difficulty in obtaining sufficient quantities of authentic standard material for analysis. Two-dimensional ^{31}P-^{1}H NMR spectroscopy has been used to identify structures of *neo*-inositol phosphates in parasitic amoebae (Martin et al., 2000), but comparisons in chemical shift to *myo*-inositol phosphates were not reported. However, the changes in chemical shifts induced by the loss of a phosphate group (e.g., from *myo*-inositol hexakisphosphate to pentakisphosphate) and by conformational changes (i.e., from the 5-axial to the 5-equatorial structure) suggest that *scyllo*, D-*chiro* and *neo* inositol hexakisphosphates will give different solution ^{31}P NMR chemical shifts to the *myo* stereoisomer.

The concentrations and proportions of *myo*-inositol hexakisphosphate measured here are similar to those reported in other studies of temperate pasture soils. For example, Oniani et al. (1973) reported inositol penta- and hexakisphosphates concentrations between 40 and 83 mg P kg^{-1} soil (17–22% total organic P) in the surface 7.5 cm of a permanent grassland soil at Rothamsted that had received various fertilizer treatments. Similar concentrations were present in the subsoil (48–90 mg P kg^{-1} soil), although the proportion of the total organic P was greater (29–37%). Omotoso and Wild (1970) reported an inositol hexakisphosphate concentration of 39 mg P kg^{-1} soil (6.5% total organic P) in a calcareous grassland soil from southern England, although a high organic matter loam contained 357 mg P kg^{-1} soil (20.4% total organic P). In the latter study, however, only 55 and 70% of the total inositol phosphates were present as the *myo* stereoisomer.

Clearly, *myo*-inositol hexakisphosphate is an important component of the organic P in temperate pasture soils, although the concentrations and proportional contribution to the total organic P can vary widely. It should be noted, however, that the analytical techniques used by some early authors may have either overestimated or underestimated the soil inositol phosphate content (Turner et al., 2002). Underestimations were caused by incomplete recovery of inositol phosphate from anion exchange columns (Anderson, 1964; Martin, 1970), whereas overestimations resulted from the inclusion of organic P compounds other than inositol phosphates in the separated fractions (Irving and Cosgrove, 1981). These errors are obviated by the deconvolution procedure, although the contribution of *myo*-inositol hexakisphosphate to the orthophosphate monoester pool is almost certainly underestimated by degradation of some orthophosphate diesters to orthophosphate monoesters in the alkaline extraction solution (Turner et al., 2003a).

Harrison (1987) reported that approximately 90% of the variation in inositol phosphate concentrations in world soils was explained by organic P, pH and organic C, but other studies have found no such correlations (McKercher and Anderson, 1968b; Williams and Anderson, 1968; Appiah and Thomas, 1982). Similarly, we did not find that concentrations of *myo*-inositol hexakisphosphate were correlated significantly with total C, total N, clay, or microbial nutrients, despite strong correlations between these properties and P compounds that would be expected to be intimately involved with organic matter turnover, including DNA and pyrophosphate (Turner et al., 2003b). This suggests that inositol phosphates accumulate in soil at least partly independently of other organic compounds and processes controlling biological nutrient turnover. This hypothesis is supported by strong correlations between concentrations of higher inositol phosphates and the P-sorption capacity of Canadian soils (McKercher and Anderson, 1968b) and between the concentrations of *myo*-inositol hexakisphosphate and amorphous Al and Fe in soils of the current study. As inositol phosphates dominate the organic P in most soils, this may also explain discrepancies in soil C, N, P, S ratios (McGill and Cole, 1981). Interestingly, the proportions of orthophosphate monoesters in humic acids of lowland rice soils of the Philippines were positively correlated with those of aromatic-C and heterocyclic-N compounds, suggesting that inositol phosphates were contained within strongly humified structures (Mahieu et al., 2002).

Despite the apparent recalcitrance of inositol phosphates in soils, the correlations between *myo*-inositol hexakisphosphate and (i) plant-available inorganic P (positive) and (ii) the total C to organic P and total N to organic P ratios (negative) indicate that *myo*-inositol hexakisphosphate con-

centrations were smallest where soil P is scarce and, presumably, the demand for P is great. This suggests that biological P availability may, in part, regulate *myo*-inositol hexakisphosphate concentrations in soils, perhaps because organisms capable of utilizing recalcitrant inositol phosphates are favored under P-limited conditions. Clearly, inositol phosphates cannot be disregarded as a biological source of soil organic P.

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AQ1 = "Can this be completed now?"