Quantification and bioavailability of *scyllo*-inositol hexa*kis*phosphate in pasture soils

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Most soils contain *scyllo*-inositol phosphates, yet quantitative data is scarce due to the analytical difficulties involved in their determination in soils. We used the recent identification of *scyllo*-inositol phosphates in alkaline soil extracts by solution ^{31}P NMR spectroscopy to re-analyze spectra from two published studies. Our aim was to quantify *scyllo*-inositol hexa*kis*phosphate in permanent lowland pasture soils and to assess its potential bioavailability to growing plants.

The first study involved twenty-nine permanent pasture soils from England and Wales (Turner *et al*., 2003). The soils were acidic (pH 4.4–6.8) with relatively large amounts of clay (22–68%), and contained a wide range of carbon (2.9–8.0%), nitrogen (0.28–0.87%), and phosphorus (0.04–0.20%). The second study involved a pot experiment with six New Zealand grassland soils in which either ryegrass (*Lolium perenne* L.) or pine seedlings (*Pinus radiata* D. Don) were grown for 10 months (Chen *et al*., 2004). The soils were acidic (pH 5.2–6.5) with relatively small amounts of clay (11–26%), but contained a wide range of carbon (3.9–9.3%), nitrogen (0.31–0.85%), and phosphorus (0.04–0.27%). In both studies, phosphorus was extracted in a solution containing 0.5 M NaOH and 50 mM EDTA (ethylenediaminetetraacetate) and analyzed by solution ^{31}P NMR spectroscopy. We identified *scyllo*-inositol hexa*kis*phosphate in re-analyzed spectra as the signal appearing close to 4.2 ppm following spectral deconvolution. Concentrations were calculated by multiplying the proportion of the spectral area assigned to *scyllo*-inositol hexa*kis*phosphate by the total phosphorus concentration of the initial extract.

Concentrations of *scyllo*-inositol hexa*kis*phosphate in twenty-nine temperate pasture soils from England and Wales ranged between 11 and 130 mg P kg^{-1} soil and accounted for between 4 and 15% of the soil organic phosphorus. Concentrations of *scyllo*-inositol hexa*kis*phosphate were correlated positively with those of organic phosphorus and *myo*-inositol hexa*kis*phosphate and negatively with the carbon-to-organic phosphorus and nitrogen-to-organic phosphorus ratios ($p<0.001$). Concentrations were also positively correlated with $NaHCO_3$-extractable organic phosphorus ($p<0.01$).

In a 10 month pot experiment with six grassland soils from New Zealand, growth of pine seedlings decreased *scyllo*-inositol hexa*kis*phosphate concentrations by between 10 and 46%. Growth of ryegrass decreased *scyllo*-inositol hexa*kis*phosphate in three low-nutrient soils by 5–21%, but increased it in three other soils by 11–16%. The different responses to the growth of ryegrass appeared to be linked to nutrient status, because decreases in *scyllo*-inositol hexa*kis*phosphate occurred in soils with smaller nutrient concentrations (nitrogen ≤0.38%;

organic phosphorus ≤0.05%), whereas increases occurred in soils with larger nutrient concentrations (nitrogen ≥0.48%, organic phosphorus ≥0.06%).

scyllo-Inositol hexa*kis*phosphate is therefore an important component of soil organic phosphorus with potential ecological significance. Inositol phosphates are stabilized strongly in soils through interaction with clay, organic matter, and metal oxides. The strong stabilization of inositol phosphates in soil also renders them relatively unavailable to plants and microorganisms, while *scyllo*-inositol hexa*kis*phosphate is expected to be especially inaccessible due to it resistance to hydrolytic attack by phytase. However, our results indicate that *scyllo*-inositol hexa*kis*phosphate is bioavailable under certain conditions, as reported previously for *myo*-inositol hexa*kis*phosphate. Decomposition by pine seedlings was mediated presumably by ectomycorrhizal fungi, which form strong associations with pine roots and secrete extra cellular phosphatases enzymes that can catalyze the hydrolysis of inositol phosphates.

Results from both re-analyzed datasets provide tentative evidence that nutrient status regulates *scyllo*-inositol hexa*kis*phosphate in soil. In particular, it was inversely correlated with nitrogen-to-organic phosphorus ratios and was degraded by ryegrass only in low-nutrient soils. This suggests that phosphorus limitation favours organisms that can access recalcitrant inositol phosphates in the soil, such as *Aspergillus ficuum* or *Pseudomonas* spp. Conversely, accumulation of *scyllo*-inositol hexa*kis*phosphate following the growth of ryegrass in high nutrient soils indicates synthesis by microbes under phosphorus-sufficient conditions.

In summary, *scyllo*-inositol hexa*kis*phosphate was confirmed as a large and potentially bioavailable component of the organic phosphorus in temperate pasture soils. Analysis of a broader range of soils and detailed mechanistic experiments are now required to probe the origins and function of this intriguing compound.

References

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