Interactions between phytase and soil constituents: implications for hydrolysis of inositol phosphates

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A large proportion of endogenous soil P occurs in organic forms, of which derivatives of inositol phosphates constitute a major fraction (Richardson *et al*., 2005). The bioavailability of inositol phosphates depends on their mineralization by extracellular phytases (*myo*-inositol-hexa*kis*phosphate phosphohydrolases; EC 3.1.3.8 and EC 3.1.3.26), which have many biological sources. However, we currently have a poor understanding of the interactions between phytases and inositol phosphates in soil. While availability of inositol phosphates is clearly a major issue, factors affecting the stability and efficacy of extracellular enzymes in soils are important. These include: (i) proteinase and microbially-mediated degradation, (ii) partial or total deactivation by adsorption onto soil particles and (iii) deactivation via interaction with polyvalent anions, metal ions and microbial metabolites (Quiquampoix and Mousain, 2005). These factors differentially affect phytase activity depending on both the characteristics of the soil environment and the source and biochemical properties of the discrete phytase.

Adsorption of enzymes to soil solid constituents is frequently reported, and demonstrated to be a result of both enthalpic (intermolecular) and entropic (intramolecular) forces (Quiquampoix, 2000). Such adsorption tends to reduce enzyme affinity for substrates and thus reduce their effective activity, which in some cases may be complete and irreversible (Quiquampoix and Mousain, 2005). However, adsorption may be important for the long-term persistence of enzymes in soils, by protecting them against degradation. Generally, it is evident that heterogenous surfaces such as those found in soils, are less inhibitory to enzyme activity upon adsorption than pure clays or some organic materials (Rao *et al.*, 1994). Moreover, inhibition upon adsorption of phytase has been shown to be soil specific, particularly in terms of soil texture and mineralology. For example, after being adsorbed for 28 days, more than 40% of added phytase remained active in a sandy soil compared to just 5% in a soil dominated by clay (George *et al*., 2005a).

The inhibition of phytase upon adsorption also appears dependent on the evolved function of the phytase. For example, intracellular phytases have been shown to be completely adsorbed and inhibited by clay particles, while extracellular phytases retained significant catalytic activity in the presence of clay (Matumoto-Pintro and Quiquampoix, 1997). Species specific variability in the biochemical properties of the extracellular enzymes produced is also important. Phosphohydrolases of one ectomycorrhizal fungus (*Pisolithus tinctorius*) showed no adsorption on montomorilonite, while those of other strains (*Cenococcum geophilum, Hebeloma cylindrosporum*) were strongly adsorbed and inhibited (Quiquampoix and Mousain, 2005).

Regardless of whether extracellular phytases are inhibited by adsorption, immobilisation to the solid phase will presumably limit their interaction with inositol phosphates in soil, particularly if these substrates are also immobilised through adsorption and precipitation reactions. With this in mind, we have observed an effect of the rhizosphere on phytase adsorption, in that greater activity was retained in rhizosphere soil solution compared to bulk soil (George *et al.*, 2005a). This might be attributed to the

changed biochemical environment in the rhizosphere, most notably modified pH or the presence of organic anions and proteins competing for adsorption sites. Changes in rhizosphere pH may also affect the adsorption reactions of phytase in soils. Phytase collected from the roots of plants expressing a phytase gene (*phyA*) from *Aspergillus niger*, was completely adsorbed in a range of soils at pH 4.5. In contrast, adsorption was decreased with increasing pH such that all remaining activity was recovered in solution at pH 7.5 (George *et al.* 2005a). Partitioning of enzyme activity to the solution phase with increased pH is attributed to enthalpic forces causing greater electrostatic repulsion above the isoelectric point (pI) of the protein, which for the *Aspergillus* phytase was pH 4.8. Importantly, when adsorption characteristics of two phytases with different pI were compared in acidic soils, major distinctions were observed. Phytase with a relatively low pI (pH 3.6 from *Peniophora lycii*) remained in solution in a range of soils (pH 4.5 to 5.0), whereas phytase with a higher pI (pH 4.8; from *Aspergillus niger*) was totally adsorbed. Moreover, we have found that the phytase with low pI is approximately twice as effective in hydrolysing inositol phosphates in soil suspensions.

The biochemical characteristics of phytases produced by different organisms have obvious implications for their behaviour in soil environments and thus their efficacy for mineralization of inositol phosphates. Maintaining phytases in solution apparently enhance their ability to interact with immobilized substrates. Therefore, increasing pH in acidic soils could be a useful management tool for making phytase more effective in the soil environment. In fact, we have recently shown that the P nutrition of transgenic plants that exude phytase was greatest in soils at neutral pH, where the ability of inositol phosphates and phytases to interact would likely be optimized (George *et al.*, 2005b). Future work will concentrate on expressing genes in plants for the production of phytases with biochemical traits that effect their interaction with soil, such as low pI. This will allow better understanding of the factors that contribute to the biological availability of inositol phosphates in soil and potentially improve the ability of plants to acquire P from this source.

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