High-performance chromatographic separations of inositol phosphates and their detection by mass spectrometry

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Mass spectrometry would appear to be an attractive approach for measuring inositol phosphates in soil, water and plant tissue. These compounds are difficult to detect by conventional UV-Visible spectroscopy because they contain no chromophoric groups. The common approach is to isolate inositols, oxidize them to release phosphate, and then detect the released phosphate colorimetrically after reaction with molybdate. While this approach is relatively simple and straightforward, it is tedious, time-consuming and subject to interferences. It is also unsuitable for on-line detection in high performance liquid chromatography separation techniques which can distinguish degrees of phosphorylation and isomeric forms of inositol phosphates.

We have shown that inductively coupled plasma (ICP) ionization and high-resolution mass spectrometry (HRMS) is superior to the colorimetric technique for quantifying organic P compounds in water (Cooper *et al.*, 2005). Detection limits are lower, analytical sensitivities are higher, and the technique can be used for both on-line and off-line chromatographic detection. We also demonstrated that electrospray (ES) ionization combined with high- and ultrahigh-resolution mass spectrometry can be used for qualitative identification of organic P species (Llewelyn *et al.*, 2002); that is, identification of molecular masses and molecular formulas of individual organic P compounds.

We have now turned our attention to coupling liquid chromatography separations with ICP and ES ionization mass spectrometry for detection of individual inositol phosphates. High performance size exclusion chromatography (HP-SEC), ion pairing reversed phase liquid chromatography (HP-IP-RPLC), and β-cyclodextrin bonded reversed phase liquid chromatography (HP-β-RPLC) have all been evaluated for their ability to separate inositol phosphates based on degree of phosphorylation and isomeric forms of individual inositol phosphates. Unfortunately, none of these techniques can be fully optimized for inositol phosphate separation because of the limitations imposed by the ICP and ES ionization steps required for mass spectrometry detection. These ionization techniques require low concentrations of organic modifiers and volatile salts in the spray matrices, greatly limiting the separation potential of the liquid chromatography methods. To date, HP-SEC has proven to be the most versatile if least efficient separation method. Resolution of all six inositol phosphates in less than 30 minutes is possible in a mobile phase that allows efficient ionization by either ICP or ES.

In previous studies of dissolved organic P speciation in the Everglades we noted that ES ionization was not very efficient for organic P. We therefore carried out a series of optimization experiments in solutions that produced acceptable separations by the HP-SEC method, using a mixture of inositol monophosphate (IP_1; $[C_6H_6(H_nPO_4)_1]$) and inositol hexa*kis*phosphate (IP_6, $[C_6H_6(H_nPO_4)_6]$. We found that the inositol phosphates were

efficiently ionized, but were surprised to observe significant fragmentation of inositol hexa*kis*phosphate, with the 2-5 congeners also appearing in the mass spectrum, as well as some sodium adducts (Figure 1). We believe that the phosphate groups in inositol phosphates may be relatively labile, and intact molecular ions may not be very abundant. Nevertheless, these results are encouraging, because they indicate that, with sufficient analytical separation of the individual inositol phosphate species, their molecular formulas can be verified by ESI-MS.

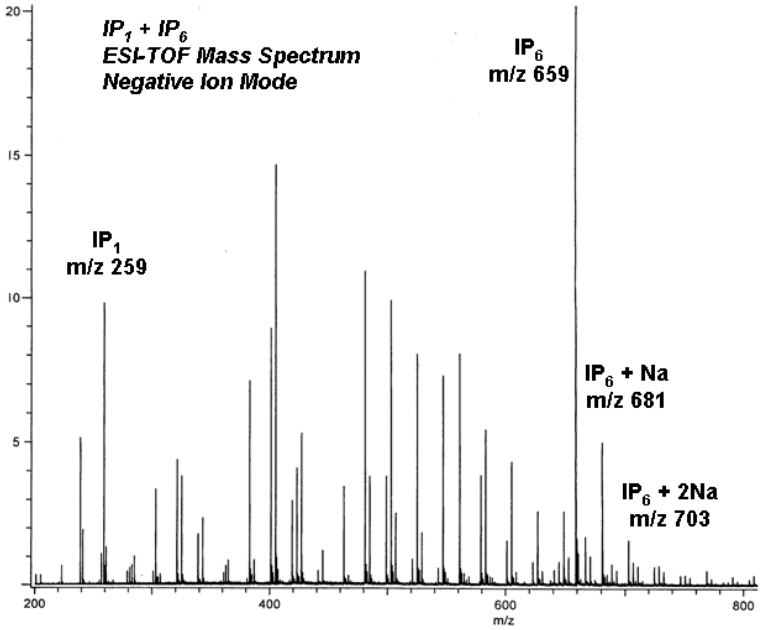

Figure 1. ESI-TOF mass spectrum of a mixture of *myo*-inositol monophosphate (IP_1) and *myo*-inositol hexakisphosphate (IP_6).

HP-SEC chromatograms of inositol phosphates with both ICP-HRMS and ES-TOF MS detection will be the primary focus of this presentation. We will also describe our efforts to further develop the chromatographic separation, using a β-cyclodextrin bonded reverse phase column and micellar mobile phase that has been shown to be effective at separating various isomeric forms of the individual inositol phosphate congeners (Dragani *et al.*, 2004).

References

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