Identification of inositol phosphates by nuclear magnetic resonance spectroscopy: unraveling structural diversity

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Inositol phosphates are widely distributed in nature. The discovery that they play critical roles in signal transduction and calcium regulation triggered widespread interest in the structures and metabolism of inositol phosphates. This interest has necessitated the need for new methods of separation and structural analysis.

Inositol is a deceptively simple molecule; the inherent stereochemical complexity of inositol and its derivatives results in multiple closely related stereo- and regioisomers. Of the nine isomers of inositol, the *myo*-isomer is the most prevalent; a total of 63 isomers of *myo*-inositol phosphates, inositol monophosphate to inositol hexa*kis*phosphate, are possible. Therefore, there is a need to distinguish between closely related isomers. An additional complexity is the fact that the metabolism of inositol phosphates are highly interconnected, changes in the concentration of one compound is rapidly transmitted to other phoshorylated derivatives by the quick action of phosphatases and kinases. Establishing the structures of inositol phosphates has been a challenge because of the need to separate and identify the structures of individual inositol phosphates in mixtures containing closely related stereo- and regioisomers.

In classic work, structures of inositol phosphates were established by first purifying the compound (HPLC, column chromatography, or high voltage paper electrophoresis) and then subjecting the purified compound to elaborate chemical and enzymatic reactions and identifying the product(s) by comigration with standards. Such indirect methods of analysis are elaborate and time consuming and provide ambiguous evidence. Thus, there is a need for techniques that allow the structural assignment of individual inositol phosphates in mixtures without prior separation.

Nuclear magnetic resonance (NMR) spectroscopy has been extensively used in the study of inositol phosphates because it can rapidly provide conclusive evidence. ^{1}H and ^{31}P NMR spectroscopic techniques are the methods of choice because of the high natural abundance and intrinsic sensitivity of the nuclei. 1-D and 2-D NMR techniques have been employed in numerous investigations by many investigators including structure determination of individual inositol phosphates in extracts of mutant corn and barley without prior separation, conformational analysis on inositol phosphates, quantitation of inositol phosphates, determination of pKa values and, more recently, solid state NMR techniques. An overview of the NMR spectroscopic techniques employed for structural analysis of inositol phosphates, the advantages and disadvantages of the various techniques and current challenges will be presented.