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A new approach to measuring phosphoinositides in cells by mass spectrometry☆

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A B S T R A C T

The phosphoinositide family of phospholipids, defined here as PtdIns, PtdIns3P, PtdIns4P, PtdIns5P, PtdIns(3,4)P_2, PtdIns(3,5)P_2, PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, play pivotal roles in organising the location and activity of many different proteins acting on biological membranes, including those involved in vesicle and protein trafficking through the endolysosomal system and receptor signal transduction at the plasma membrane. Accurate measurement of the cellular levels of these lipids, particularly the more highly phosphorylated species, is hampered by their high polarity and low cellular concentrations. Recently, much progress has been made in using mass spectrometry to measure many different lipid classes in parallel, an approach generally referred to as 'lipidomics'. Unfortunately, the acidic nature of highly phosphorylated phosphoinositides makes them difficult to measure using these methods, because they yield low levels of useful ions; this is particularly the case with PtdIns(3,4,5)P_3. We have solved some of these problems by methylating the phosphate groups of these lipids with TMS-diazomethane and describe a simple, integrated approach to measuring PtdIns, PtdInsP, PtdInsP_2 and PtdInsP_3 classes of lipids, in parallel with other phospholipid species, in cell and tissue extracts. This methodology is sensitive, accurate and

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robust, and also yields fatty-acyl compositions, suggesting it can be used to further our understanding of both the normal and pathophysiological roles of these important lipids.

Introduction

Inositol lipids, or phosphoinositides, comprise a distinct family of eight phospholipids, one or more of which are found in all eukaryotic cells; these are PtdIns, PtdIns3P, PtdIns4P, PtdIns5P, PtdIns(3,4)P_2, PtdIns(3,5)P_2, PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 (Fig. 1). They are interconverted by lipid kinases, phosphatases and phospholipases which acutely regulate their levels in response to different environmental cues. Several, perhaps all, of these lipids play major regulatory roles in cells by dictating the localisation and function of proteins which act on the membrane in which they reside (Di Paolo and De Camilli, 2006). In most examples studied to date, this involves the specific recognition of a phosphorylated inositol headgroup by a conserved protein domain, for example the binding of the PtdIns(4,5)P_2 or PtdIns(3,4,5)P_3 headgroups by distinct families of pleckstrin homology (PH) domains (Lemmon, 2008). Moreover, at least two of these lipids are involved in reactions which represent the rate limiting steps in the generation of 'second-messengers' in response to activation of cell surface

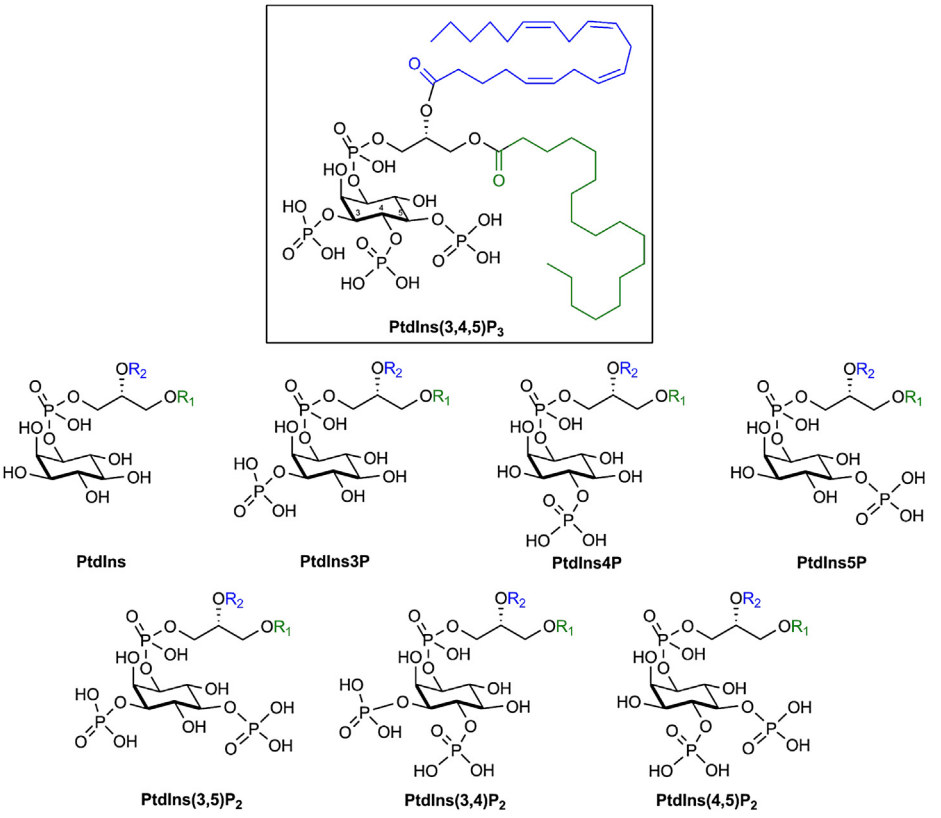

Fig. 1. The structures of phosphoinositides found in eukaryotic cells. The structure of PtdIns(3,4,5)P_3 is shown with 18:0 (stearoyl, green) and 20:4 (arachidonoyl, blue) acyl groups at the *sn*-1 and *sn*-2 positions, respectively, as an example of the major molecular species found in mammalian cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)