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OPINION PAPER

The physical influence of inositides—a disproportionate effect?

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Abstract After the initial observation that lipids form a considerable part of biological membranes, the details of the physical role of lipids in biological systems have emerged gradually. There have been few 'Eureka' moments in which a class or individual lipid has appeared as a game-changing physical player. However, evidence collected in the last five years suggests that that notion may be about to change. In chemical biology studies, inositides are increasingly showing themselves to be lipids that have a physical influence on membrane systems that is as strong as their biological (signalling) one. Additionally, recent evidence has shown that the concentration of at least one inositide changes during important stages of the cell cycle, and not in a manner consistent with its traditional signalling roles. The balance between these data is explored and a forward-looking view is proposed.

Keywords Inositides · Lipid phase behaviour · Lipid biophysics

Inositides have been recognised for some time as important biological signalling molecules. Work by Berridge and Irvine in the 1980s demonstrated their role as secondary messengers [1]; since then, it has become clear that the range of cellular processes controlled by inositides in mammals is considerable. The best known inositides are probably phosphatidylinositol-3,4,5-trisphosphate (PIP3, see Fig. 1) and phosphatidylinositol-4,5-phosphate (PIP2, Fig. 1). PIP2, along with phosphatidylinositol (PI) and phosphatidylinositol 4-phosphate (PI-4-*P*) make up the bulk of inositide lipids in vivo [2, 3]. The biological importance of PIP2 and PIP3 is exemplified by the effect of mutations in the proteins that convert the former into the latter. Such mutations in one protein complex (PI3K) alone form the basis of the molecular mechanisms behind multiple sclerosis [4], respiratory inflammation in asthma [5], schizophrenia [6], and a variety of cancers [7], as well as in the normal function of leukocytes [8].

The apparent ubiquity of these two inositides in mammalian systems has aroused research interest from several angles. There is a mounting body of evidence that it is not only mammals that make use of PIP2 as a signalling molecule, but also insects [9] and plants [10]. Several studies have suggested that PIP2 has a physical role that might be quite separate from its biological one. The particular processes in which PIP2 is a protagonist include membrane trafficking [11, 12], vesicle formation [12, 13], clatharin-mediated endocytosis [13, 14], and in membrane dynamics [15]. There is also some indication that the activity of phospholipase Cγ, for which PIP2 is a substrate, may have a physical role in membrane fusion [16]. It also seems likely that PIP2 is a team player, acting with other lipids such that certain physical processes may be completed. A study of pollen tubes in *Arabidopsis* provided evidence that a particular balance between PIP2 and PI-4-*P* is required for endocytosis to be successful [17]. There have not yet been any comprehensive studies of the phase behaviour of PIP2, and thus, it is not yet clear whether it is typically a bilayer-forming lipid or one that has an energetic preference for a curved topology.

Despite the paucity of phase behaviour data on PIP2, the phase behaviour of the physiological precursor to PIP2, PI-4-*P*, has been researched. Furse et al. reported that PI-4-*P* is able to induce an inverse hexagonal phase in the typically-lamellar PC, at 2 mol % [18], strongly suggesting that PI-4-*P* is not a

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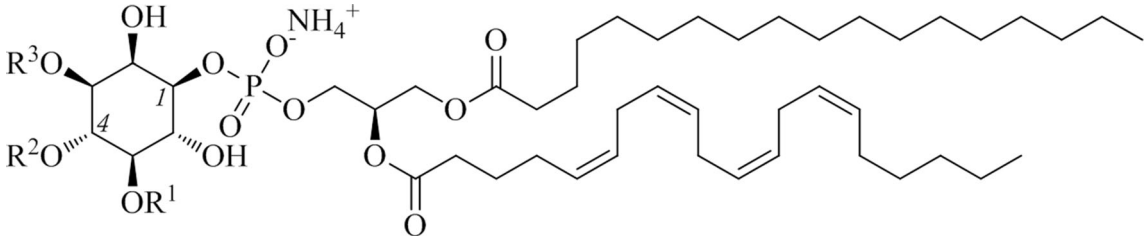

Fig. 1 The structure of the inositide lipid group. All combinations and permutations of phosphates at R^1, R^2 and R^3 have been found in vivo, with phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2; R^2 and R^3 = -PO_3H^-, R^1 = H), phosphatidylinositol 4-phosphate (PI-4-*P*; R^2 = -PO_3H^-, R^1 and R^3 = H), and phosphatidylinositol (PI, R^1, R^2 and R^3=H) forming the bulk of the mass of inositides under typical physiological conditions [2, 3]

bilayer-forming lipid. The inverse hexagonal phase was observed over a wide temperature and pressure range at physiological *p*H and hydration. The evidence from this study is striking because 2 mol % is a typical physiological concentration for this lipid [3, 19]. There is now evidence that a physiological concentration of PI-4-*P* is required for the flippase activity of Drs2p on PS. Importantly, the PI-4-*P* does not appear to behave like a signalling species in this process.

The physical behaviour of PI-4-*P* is not dissimilar to some of that observed of the physiological precursor to PI-4-*P*, PI. Mulet et al. reported that in a system comprising 10 mol % naturally occurring PI in phosphatidylcholine (PC) at 30–40 wt% hydration, the fluid lamellar phase was essentially part of the transition to a curved phase that possessed *Fd3m*-geometry, rather than the most thermodynamically stable phase [20]. The *Fd3m* geometry is the most curved assembly of lipids yet recorded, and is relatively uncommon in phospholipid systems [21, 22]. Work carried out by Larijani and Dufourc, using deuterium NMR, suggests the presence of an isotropic phase at 20 % PI in PC that is consistent with a cubic phase that appears to be temperature dependent [23].

The observations about the behaviour of PI are important because they challenge the idea that PI is exclusively a bilayer-forming lipid, implying that PI is a source of curvature elastic stress at physiological concentrations. This has far-reaching consequences, as PI is the commonest inositide, forming an important fraction of animal [2, 3, 24], plant [25], and some bacterial [26] membranes. This understanding of the physical behaviour of PI has recently been shown in a new light by a recent report from Atilla-Gokcumen et al. about the lipid composition of HeLa cells [24]. These researchers provide compelling evidence that the concentration of PI increases by an order of magnitude in the plasma membrane of HeLa cells during cytokinesis. This suggests that not only is the presence of PI important for the process of cell division, but also, probably, in a way that is not restricted to its role as a precursor for inositide signalling molecules. The increase in the concentration of during cytokenesis also implies an active regulation of particular lipid species during division.

Furthermore, the physical role of inositides may not be restricted simply to their being present in a given membrane. Evidence that indicates the role of derivatives of inositides, such as diglycerides (DAGs), is accumulating. DAGs appear to play a crucial role in the formation for the nuclear envelope and in directing the morphology of the endoplasmic reticulum [26, 27]. Inositide–protein interactions may also be important for structural reasons on a molecular scale. Evidence for a role of phosphorylated inositides as stabilisers of protein structure has also begun to emerge [28]. Lagonowsky et al. tested a number of lipids, including PC, PE, CL, PG, PS, and PA, and report that phosphatidylinositol (PI-3-*P*) binds the most strongly to folded membrane proteins, and in a manner that prevented the proteins from unfolding in the gas phase used in ion mobility mass spectrometry.

Comparing the biological and physical data about inositides gives us a tantalising picture of their general role in vivo. It is clear that inositides, or at least those that represent the bulk of that fraction (PI, PI-4-*P* and PIP2) have an active role in a number of significant membrane events, such as membrane division. Whether inositides form part of a direct physical driving force for such processes is not yet clear, but it seems that hypotheses based on inositides as inert bystanders or lipids that possess only a biological role, are no longer tenable.

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