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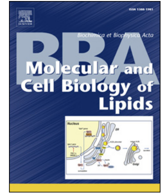

Review

PI(4,5)P_2-binding effector proteins for vesicle exocytosis ☆

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ABSTRACT

PI(4,5)P_2 participates directly in priming and possibly in fusion steps of Ca^{2+}-triggered vesicle exocytosis. High concentration nanodomains of PI(4,5)P_2 reside on the plasma membrane of neuroendocrine cells. A subset of vesicles that co-localize with PI(4,5)P_2 domains appear to undergo preferential exocytosis in stimulated cells. PI(4,5)P_2 directly regulates vesicle exocytosis by recruiting and activating PI(4,5)P_2-binding proteins that regulate SNARE protein function including CAPS, Munc13-1/2, synaptotagmin-1, and other C2 domain-containing proteins. These PI(4,5)P_2 effector proteins are coincidence detectors that engage in multiple interactions at vesicle exocytic sites. The SNARE protein syntaxin-1 also binds to PI(4,5)P_2, which promotes clustering, but an activating role for PI(4,5)P_2 in syntaxin-1 function remains to be fully characterized. Similar principles underlie polarized constitutive vesicle fusion mediated in part by the PI(4,5)P_2-binding subunits of the exocyst complex (Sec3, Exo70). Overall, focal vesicle exocytosis occurs at sites landmarked by PI(4,5)P_2, which serves to recruit and/or activate multifunctional PI(4,5)P_2-binding proteins. This article is part of a Special Issue entitled Phosphoinositides.

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1. Introduction

Early studies showed that Ca^{2+}-triggered vesicle exocytosis in permeable neuroendocrine cells requires MgATP for a priming step that precedes Ca^{2+}-triggered fusion [1,2]. The MgATP-dependent priming step involves phosphoinositides [3] and requires cytosolic factors (phosphatidylinositol transfer protein and phosphatidylinositol(4) phosphate 5-kinase, PI(4)P 5-kinase) [4,5], which indicates that PI(4)P phosphorylation to PI(4,5)P_2 is essential for maintaining regulated exocytosis. This was further shown with a high affinity PI(4,5)P_2-binding pleckstrin homology domain (PH) from PLCδ_1 [6] and by the enzyme-catalyzed hydrolysis of PI(4,5)P_2 [5,7,8], which inhibit evoked vesicle exocytosis in neuroendocrine cells. Evoked exocytosis was also shown to be inhibited by the small HIV-1 Tat protein, which directly enters cells and binds PI(4,5)P_2 with ~20-fold greater affinity than the PLCδ_1 PH domain [9,10]. Similarly, a mouse knockout for PI(4)P 5-kinase Iγ caused a reduction in the priming of neuronal dense-core vesicles [11]. Conversely, increased synthesis of PI(4,5)P_2 by PI(4)P 5-kinase activation increases sustained rates of evoked secretion [8,12]. Phospholipase C-catalyzed inhibition of exocytosis in permeable cells [5] suggested that there was no major role for PI(3,4,5)P_3 (but see below), which was reinforced by the lack of inhibition of ATP-dependent priming by LY294002, a PI 3-kinase inhibitor, [13]; however, inhibition of evoked exocytosis by LY294002 was reported in other studies [14] but this compound also inhibits type III PI 4-kinases [15]. Electrophysiological studies of evoked vesicle exocytosis in neuroendocrine cells showed that PI(4,5)P_2 is required for the generation of a primed pool of ready-releasable vesicles as well as for sustained secretion, which represents priming of recruited vesicles [8,16]. These studies strongly indicate an essential role for PI(4,5)P_2 in priming reactions for vesicle exocytosis but they do not exclude additional roles for PI(4,5)P_2 at later steps following priming (e.g., fusion). Moreover, this work did not elucidate the precise roles for PI(4,5)P_2, which has been the major focus of more recent research.

PI(4,5)P_2 as a signaling molecule is abundant in the inner leaflet of the plasma membrane (2 mol%) but much sparser in intracellular membrane compartments. The intact phosphoinositide PI(4,5)P_2 plays a critical role in most if not all cellular events associated with the plasma membrane including regulated vesicle exocytosis [17], constitutive vesicle exocytosis [18], endocytosis [19], F-actin assembly [20], cell adhesion [21], phagocytosis [22], viral budding [23], enzyme activation [24], ion channel regulation [25] and cytokinesis [26]. PI(4,5)P_2 serves as a marker for plasma membrane identity and establishes a landmark for plasma membrane-associated cellular events [27]. The landmark role for PI(4,5)P_2 is interpreted through the interactions of PI(4,5)P_2 with proteins that are involved in each of the above cellular processes. Protein binding to PI(4,5)P_2 occurs either through structured domains that have basic charge regions such as PH and C2 domains, or through

Abbreviations: PI, phosphosphatidylinositol; PI(4)P, phosphatidylinositol 4-monophosphate; PI(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; PKC, protein kinase C; TIRF, total internal reflectance fluorescence; PALM, photoactivated localization microscopy; STORM/dSTORM, stochastic optical reconstruction microscopy/direct; STED, stimulated emission depletion microscopy; FWHM, full width half maximum; MARCKS, myristoylated alanine-rich C kinase substrate; CAPS (aka CADPS), Ca^{2+}-dependent activator protein for secretion; Munc13, mammalian Unc13 protein; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; VAMP-2 (aka synaptobrevin-2), vesicle-associated membrane protein-2; GRP1, general receptor of phosphoinositides

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contiguous or non-contiguous basic charge clusters on proteins [28–30]. $PI(4,5)P_2$-binding proteins are the effectors for the biological roles of $PI(4,5)P_2$ where $PI(4,5)P_2$ functions as a co-factor either to activate membrane proteins or to recruit proteins to the plasma membrane for function. $PI(4,5)P_2$ effector proteins are commonly multi-domain coincidence detectors that exhibit interactions with $PI(4,5)P_2$ and with other membrane constituents. Membrane-binding energies sum from multiple low affinity interactions to drive high affinity membrane binding. Especially for Ca^{2+}-dependent membrane binding, there is a marked mutual synergy among interaction partners. For vesicle fusion at the plasma membrane, there are now several examples of $PI(4,5)P_2$-binding proteins that interact with SNARE proteins to promote their assembly for membrane fusion. Multivalent protein–lipid and protein–protein interactions result in higher affinity binding to the plasma membrane and provide orientation of the recruited protein relative to membrane sites landmarked by $PI(4,5)P_2$.

Recent work has characterized the unique distribution of $PI(4,5)P_2$ in the inner leaflet of the plasma membrane in neuroendocrine cells that contribute to establishing sites for vesicle exocytosis. Increasingly, the $PI(4,5)P_2$ effector proteins involved in vesicle exocytosis have been characterized for their recruitment to or activation at sites of exocytosis. TIRF (total internal reflectance fluorescence) and super-resolution microscopy have played an increasing role in establishing the lipid and protein distribution at sites of exocytosis. A number of recent reviews have summarized the proteins and lipids involved in vesicle exocytosis [31–40].

2. $PI(4,5)P_2$ localizes to sites of vesicle exocytosis

While a role for $PI(4,5)P_2$ in vesicle exocytosis has been established, it is important to determine whether regulation by $PI(4,5)P_2$ is local or distant, and whether $PI(4,5)P_2$ localizes to sites of exocytosis. Early studies [3,5] indicated that $PI(4,5)P_2$ hydrolysis to diffusible products did not mediate the essential role of $PI(4,5)P_2$ in vesicle exocytosis suggesting that regulation was local. The current technology is limited for localizing and perturbing $PI(4,5)P_2$ although this is improving [41–43]. Several studies revealed a heterogeneous distribution of $PI(4,5)P_2$ on the plasma membrane of neuroendocrine and other cells. Caroni and coworkers reported that immunoreactive clusters of $PI(4,5)P_2$ were evident in fixed PC12 cells [44] using a $PI(4,5)P_2$ antibody [45]. Because fixation does not immobilize lipids, it was possible that bivalent antibodies induce $PI(4,5)P_2$ clustering, but a number of cellular conditions were found that alter the size of the clusters (e.g., MARCKS overexpression) suggesting the clusters were physiological. Several studies showed that diffraction-limited puncta of $PI(4,5)P_2$ could be imaged on plasma membrane sheets derived from PC12 or chromaffin cells [8,46,47]. These $PI(4,5)P_2$ puncta were similarly imaged either with $PI(4,5)P_2$ antibody [8,46] or with a GFP fusion of the PH domain of $PLC\delta_1$ [46,47]. Immuno-EM studies localized $PI(4,5)P_2$ close to chromaffin granules [48]. In the studies of Aoyagi et al., ~20% of the dense-core vesicles on membrane sheets co-localized with $PI(4,5)P_2$ with about half of these also co-localizing with syntaxin-1 [46]. In the studies of James et al., ~20% of the dense-core vesicles on membrane sheets co-localized with the priming factor CAPS/CADPS and with $PI(4,5)P_2$ [47]. These studies indicate that $PI(4,5)P_2$ is distributed in microdomains on the plasma membrane of neuroendocrine cells. A subset of $PI(4,5)P_2$ microdomains co-localize with vesicles and with proteins essential for vesicle exocytosis, which indicates that $PI(4,5)P_2$ is present at sites of vesicle exocytosis (as well as elsewhere) and likely exerts local regulation.

In the studies of James et al., the $PLC\delta_1$-PH-GFP probe was calibrated on supported bilayer membranes to assess membrane $PI(4,5)P_2$ concentrations [47]. $PI(4,5)P_2$ was present in domains at ≥ 6 mol% although this was an underestimate limited by lack of knowledge of the actual size of the diffraction-limited domain. A subsequent study [49] estimated the size of $PI(4,5)P_2$ domains using STED microscopy. The $PI(4,5)P_2$ domains had an average diameter (FWHM) of 73 ± 42 nm (s.d.) enabling the authors to calculate that $PI(4,5)P_2$ concentrations at the peak of the domain may reach ~82 mol%. This may also be an underestimate based on possible crowding of the $PLC\delta_1$-PH domain interacting 1:1 with $PI(4,5)P_2$ headgroups. Nonetheless, the nanodomains of $PI(4,5)P_2$ imaged in this study [49] appear to consist of very high concentrations of $PI(4,5)P_2$ approaching 100 mol%. A study utilizing dSTORM with directly-labeled monoclonal antibodies to $PI(4,5)P_2$ in PC12 cells confirmed the small size of $PI(4,5)P_2$ nanodomains (~65 nm). From the high signal-to-noise ratio of dSTORM, the authors concluded that the majority of plasma membrane inner leaflet $PI(4,5)P_2$ was detected in nanodomain clusters [50].

As noted, $PI(4,5)P_2$ domains co-localize with only a subset of docked vesicles (~20% in membrane sheets; but see below); however, these could represent a primed subset of vesicles. This was suggested by studies in which the co-localization of $PI(4,5)P_2$ with vesicles was reduced by briefly evoking exocytosis with Ca^{2+} influx [46]. Whether evoked vesicle fusion occurs preferentially at $PI(4,5)P_2$-rich plasma membrane sites was addressed in a recent study [51]. Kabachinski et al. used a lower affinity $PI(4,5)P_2$-binding PH domain probe derived from $PLC\delta_4$ rather than $PLC\delta_1$ to image $PI(4,5)P_2$ microdomains in live PC12 cells by TIRF microscopy. As was the case for membrane lawn studies, there were a much larger number of $PI(4,5)P_2$ microdomains than membrane-proximal vesicles, which is consistent with a role for $PI(4,5)P_2$ in many membrane-linked events. However, ~50% of the dense-core vesicles in the TIRF field of live cells co-localized with the $PLC\delta_4$-PH-GFP probe. Ca^{2+}-induced vesicle exocytosis was found to occur at membrane sites enriched for $PI(4,5)P_2$ based on the $PLC\delta_4$-PH-GFP fluorescence. A PKC-C1-GFP probe detected no $PI(4,5)P_2$ hydrolysis to DAG at sites of vesicle exocytosis under Ca^{2+} influx conditions optimal for exocytosis. Greater Ca^{2+} influx did generate DAG (see below). As anticipated for the lower affinity of the $PLC\delta_4$-PH domain for $PI(4,5)P_2$ [15] as compared to a $PLC\delta_1$-PH domain probe, the $PLC\delta_4$-PH domain probe exhibited reduced partitioning onto the plasma membrane and only partially inhibited vesicle exocytosis [51]. These studies indicate that vesicle exocytosis can occur at membrane sites highly enriched for $PI(4,5)P_2$. It will be of interest to use super-resolution microscopy to determine whether $PI(4,5)P_2$ nanodomains disperse or merge with the vesicle membrane at sites of fusion. Diffusion of $PI(4,5)P_2$ onto the vesicle membrane during fusion, as shown to occur in Xenopus egg cortical granules [52], would promote re-organization of the actin cytoskeleton that could alter vesicle fusion modes or vesicle retrieval in endocytosis.

3. Basis for $PI(4,5)P_2$ cluster formation

An important but unresolved question is the basis for $PI(4,5)P_2$ micro/nanodomain formation. Early studies suggested that cellular $PI(4,5)P_2$ co-purifies with cholesterol-rich, detergent-resistant membrane domains [44,53] but other studies provided some evidence against this [46,49]. Recent super-resolution microscopy studies provide support for the concept that $PI(4,5)P_2$ clusters in the cytoplasmic leaflet align with cholesterol- and sphingomyelin(SM)-rich regions in the ectoplasmic leaflet at least at some plasma membrane sites. Kobayashi and co-workers expressed a fluorescent $PLC\delta_1$-PH domain probe to label the cytoplasmic leaflet, and used an SM-binding protein to label the ectoplasmic leaflet in PALM/dSTORM studies. They detected aligned $PI(4,5)P_2$/SM clusters that were on average ~250 nm [26]. SM clustering appeared to be required for $PI(4,5)P_2$ domain formation [26]. These studies provide evidence that cytoplasmic leaflet $PI(4,5)P_2$ domains may correspond in part to cholesterol/SM-rich raft domains.

Substantial experimental work suggests that $PI(4,5)P_2$ in synthetic membranes can self-associate in microdomains via H-bonding interactions especially when electrostatic repulsive interactions are neutralized with cations such as Ca^{2+} [54,55]. $PI(4,5)P_2$ clustering can also be achieved by electrostatic interactions between proteins with basic charge regions and highly anionic $PI(4,5)P_2$ (-4 charge at pH 7.0) [56]. The charge cluster on MARCKS peptide (KKKKKRFSFKKSFKLSGFSFKKNKK)

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