Spotlight on the Binding Affinity of Ion Channels for Phosphoinositides: From the Study of Sperm Flagellum

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The previous studies revealed that many types of ion channels have sensitivity to PtdIns(4,5)P2, which has been mainly shown using heterologous expression system. On the other hand, there remains few evidence showing that PtdIns(4,5)P2 natively regulate the ion channel activities in physiological context. Our group recently discovered that a sperm specific K+ channel, Slo3, is natively regulated by PtdIns(4,5)P2 in sperm flagellum. Very interestingly, a principal piece, to which Slo3 specifically localized, had extremely low density of PtdIns(4,5)P2 compared to the regular cell plasma membrane. Furthermore, our studies and the previous ones also revealed that Slo3 had much stronger PtdIns(4,5)P2 affinity than KCNQ2/3 channels, which are widely regulated by endogenous PtdIns(4,5)P2 in neurons. Thus, the high-PtdIns(4,5)P2 affinity of Slo3 is well-adapted to the specialized PtdIns(4,5)P2 environment in the principal piece. This study sheds light on the relationship between PtdIns(4,5)P2-affinity of ion channels and their PtdIns(4,5)P2 environment in native cells. We discuss the current understanding about PtdIns(4,5)P2 affinity of diverse ion channels and their possible regulatory mechanism in native cellular environment.

Keywords: phosphoinositides, ion channel, voltage-sensing phosphatase, sperm flagellum, Slo3, KCNQ

INTRODUCTION

Phosphoinositides (PIPs) comprise a minor proportion of the lipid membrane, but they play important roles in a variety of physiological processes, including signal transduction, regulation of cytoskeleton, exocytosis, and endocytosis (Balla, 2013). Specifically, PtdIns(4,5)P2, one class of PIPs, mainly exists in the inner leaflet of the plasma membrane. Accumulating evidence suggest that PtdIns(4,5)P2 also regulates the property of diverse ion channels in many aspects (Suh and Hille, 2005, 2008; Okamura et al., 2018). For example, PtdIns(4,5)P2 is required for the basal activities of all five members of voltage-gated potassium KCNQ/Kv7 channels (Kv7.1-7.5 or KCNQ1-5) (Zhang et al., 2003). Currently, more than 50 ion channel molecules are identified as the targets of PtdIns(4,5)P2 regulation from the electrophysiological studies using heterologous expression system (Suh and Hille, 2008; Okamura et al., 2018). In these studies, the PtdIns(4,5)P2 dependency of ion channels are examined using application of soluble short chain PtdIns(4,5)P2, activation of voltage-sensing phosphatase (VSP), or Gq-couple receptors activation. In contrast to the plenty of knowledge about ion channel regulation by PtdIns(4,5)P2 from these experiments, the number of reports about these regulatory mechanisms is quite limited in native physiological condition. The evidence of its physiological importance originates in the study of M-current in sympathetic neurons (Brown and Adams, 1980), which is now widely observed in various types of neurons. The M-current is evoked by the stimulation of muscarinic acetylcholine receptors.
(mAchRs), which activates GPCR/Gq signaling cascade and reduces the PtdIns(4,5)P$_2$ level in plasma membrane. The reduced PtdIns(4,5)P$_2$ level causes the suppression of KCNQ2/3 channel activities, inducing the depolarization of neurons. The observation of M-current in native neurons indicates that KCNQ channels is constitutively activated by PtdIns(4,5)P$_2$ in normal condition, and the change of PtdIns(4,5)P$_2$ level is also physiologically important for the neural function. Our group recently reported that Slo3, a sperm specific K$^+$ channel, is natively regulated by PtdIns(4,5)P$_2$ in sperm flagellum (Kawai et al., 2019). We analyzed the function of VSP, which dephosphorylates PtdIns(4,5)P$_2$ into PtdIns(4)P, in a mouse spermatozoa. We found that the amount of PtdIns(4,5)P$_2$ was significantly and highly upregulated in a VSP-deficient spermatozoa, thereby the activity of Slo3 was significantly increased (Figure 1A; Kawai et al., 2019), which is consistent with the previous report that Slo3 is sensitive to PtdIns(4,5)P$_2$ (Tang et al., 2010). Importantly, we observed that the principal piece, to which Slo3 specifically localizes, showed extremely low density of PtdIns(4,5)P$_2$ compared with the previously reported regular plasma membrane (Kawai et al., 2019). The Slo3 has strong binding affinity for PtdIns(4,5)P$_2$ and the low level of PtdIns(4,5)P$_2$ appears to adjust to the high-affinity of Slo3 for proper regulation (Figure 1B). The previous reports indicate that Slo3 has more than 10-fold higher affinity for PtdIns(4,5)P$_2$ than KCNQ2/3 channels and that the PtdIns(4,5)P$_2$ level in principal piece is also less than one-tenth of the regular plasma membrane as reported in fibroblast (Fujita et al., 2009). Thus, the study sheds light on the importance of focusing on the relationship between PtdIns(4,5)P$_2$-affinity of ion channels and the native PtdIns(4,5)P$_2$ level. Nevertheless, we sometimes discuss the PtdIns(4,5)P$_2$-sensitivity of ion channels in an all-or-none manner, and the affinity of PtdIns(4,5)P$_2$ is not so importantly discussed. In the present mini-review, we describe how the PtdIns(4,5)P$_2$ sensitivity or affinity of ion channels has been examined in heterologous expression system at first. Then, we introduce the list of PtdIns(4,5)P$_2$-sensitive ion channels with their affinity as described. Finally, we discuss the possibility that different PtdIns(4,5)P$_2$-affinity of ion channels are regulated in different PtdIns(4,5)P$_2$ environment of specialized subcellular compartments.

## Quantifying the Affinity of Ion Channels for PtdIns(4,5)P$_2$ Using Heterologous Expression System

The PtdIns(4,5)P$_2$-sensitivity of ion channels is also mostly examined by electrophysiology. The ion channel of interest is heterologously expressed in *Xenopus* oocyte or other cell lines such as Human Embryonic Kidney cells 293 (HEK-293) or Chinese hamster ovary (CHO) cells. There are several techniques to manipulate plasma membrane PtdIns(4,5)P$_2$ levels so that the change in ion channel activities is monitored. For example, heterologous expression of Gq-couple receptors such as muscarinic acetylcholine receptor M1 is widely used for this purpose. The agonist stimulation cleaves PtdIns(4,5)P$_2$ to reduce its level on the plasma membrane. Furthermore, a rapamycin-inducible phosphatase, Pseudojanin, strongly depletes PtdIns(4,5)P$_2$, as well as PtdIns(4)P (Hammond et al., 2012). Although these chemical techniques are important molecular tools to manipulate the PtdIns(4,5)P$_2$, it may not be suited for estimating PtdIns(4,5)P$_2$-affinity of ion channel due to the difficulty in precisely manipulating the PtdIns(4,5)P$_2$ levels.

The most quantitative method to analyze PtdIns(4,5)P$_2$-affinity is performed by directly applying a soluble short acyl chain PtdIns(4,5)P$_2$ to the inner leaflet of plasma membrane in an inside-out configuration. This method allows to describe a dose-response curve and to calculate the Kd-value, suited for discussing the PtdIns(4,5)P$_2$-affinity of ion channels. On the other hand, because this technique uses short acyl chain PtdIns(4,5)P$_2$ instead of native long acyl chain lipids, it may show some artificial effects on ion channel properties. Indeed, although the PtdIns(4,5)P$_2$ sensitivity of several K$^+$ channels has been already reported by perfusing soluble PtdIns(4,5)P$_2$, the sensitivity has not been reproduced using other techniques to deplete PtdIns(4,5)P$_2$ (Kruse et al., 2012). A possible alternative way to estimate PtdIns(4,5)P$_2$-affinity is using VSP, which dephosphorylates PtdIns(4,5)P$_2$ in response to the depolarization (Murata et al., 2005). Because VSP depletes the endogenous PtdIns(4,5)P$_2$ by its enzyme activity, it can examine the importance of the regulation by PtdIns(4,5)P$_2$ in a more physiological aspect. Although the quantitativity of VSP for PtdIns(4,5)P$_2$-affinity is not more precise than that of soluble...
PtdIns(4,5)P₂ perfusion, the strength of VSP activity can be easily controlled by changing the amplitude, duration, and/or number of the depolarization pulses, conferring a certain level of quantitativity to it. Ideally, it should be the best way to quantify the PtdIns(4,5)P₂ level of quantitativity to it. Ideally, it should be the best way to quantify the PtdIns(4,5)P₂ level of quantitativity to it. Ideally, it should be the best way to quantify the PtdIns(4,5)P₂ level of quantitativity to it. Ideally, it should be the best way to quantify the PtdIns(4,5)P₂ level of quantitativity to it. Ideally, it should be the best way to quantify the PtdIns(4,5)P₂ level of quantitativity to it. Ideally, it should be the best way to quantify the PtdIns(4,5)P₂ level of quantitativity to it. Ideally, it should be the best way to quantify the PtdIns(4,5)P₂ level of quantitativity to it. Ideally, it should be the best way to quantify the PtdIns(4,5)P₂ level of quantitativity to it. 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the flagellar PtdIns(4,5)P$_2$ environment for regulating a high-affinity Slo3 channel activity. Collectively, PtdIns(4,5)P$_2$-affinity of ion channels would be natively regulated by the change of PtdIns(4,5)P$_2$ level in plasma membrane of regular cells. However, it is noteworthy that the distribution of PtdIns(4,5)P$_2$ in plasma membrane is sometimes heterogeneous. The previous reports showed that PtdIns(4,5)P$_2$ is concentrated at the rim of caveolae, a subset type of lipid raft, in cultured fibroblasts and smooth muscle cells of a mouse using freeze-fracture replica method (Fujita et al., 2009). Furthermore, it has been recently shown that there is compartmentalization of PtdIns(4,5)P$_2$ metabolism; PtdIns(4,5)P$_2$ break-down by G protein/PLC pathway is more rapid in cholesterol-rich domain (raft domain) than in cholesterol-poor domains (non-raft domain) of the plasma membrane (Myeong et al., 2021). These lines of evidence may suggest that lipid raft structure is more suited for regulating ion channel activities for PtdIns(4,5)P$_2$ low-affinity ion channels due to the abundant PtdIns(4,5)P$_2$ level.

The level of PtdIns(4,5)P$_2$ can also be a variable depending on the subcellular compartments. For example, PtdIns(4,5)P$_2$ is enriched in dendritic spines of cultured hippocampal neurons (Horne and Dell’acqua, 2007). The specialized structures such as flagellum or cilia can also have different PtdIns(4,5)P$_2$ environment. As described above, we discovered that sperm flagellum show extremely low level of PtdIns(4,5)P$_2$ due to VSP activity. Furthermore, several reports revealed that primary cilia, a non-motile single sensory organelle, show extremely low level of PtdIns(4,5)P$_2$ due to accumulation of Inpp5e, a phosphoinositide 5-phosphatase (Chavez et al., 2015; Garcia-Gonzalo et al., 2015). Interestingly, this PtdIns(4,5)P$_2$ level can also be dynamically regulated by the depletion of Inpp5e during cell-division cycle (Phua et al., 2017). Therefore, the activity of high PtdIns(4,5)P$_2$ affinity ion channels, if there is any in primary cilia, could be regulated during the cell division cycles.

Overall, the different PtdIns(4,5)P$_2$-affinity among ion channels may implicate that their regulation by PtdIns(4,5)P$_2$ is dependent on the individual PtdIns(4,5)P$_2$ environment, although it is also possible that PtdIns(4,5)P$_2$-affinity have the other significance than regulation of ion channel activity, such as localization and trafficking of ion channels (Van Den Bogaart et al., 2011). In any case, it is important to focus on the PtdIns(4,5)P$_2$-affinity, as well as the spatial information, to precisely examine the ion channel function in the future.

**AUTHOR CONTRIBUTIONS**

TK and YO wrote the draft of the manuscript and approved the submitted version.

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