The presence of PI-rich vesicles is required for the PLC ζ activation according to mathematical modeling

by A.K. Tabachnik^{1#}

1. Faculty of Physics, Lomonosov Moscow State University, 1/2 Leninskie gory, Moscow 119991, Russia

Correspondence: atabachnick@mail.ru

Received: 25.06.2021

Accepted: 29.06.2021

Published: 30.06.2021

DOI: 10.52455/sbpr.01.202102013

Abstract

Phospholipase C ζ (PLC ζ) is an enzyme found in the cytoplasm and acrosome of mammalian spermatozoa. It catalyzes the reaction of phosphatidylinositol-4,5-phosphate hydrolysis into inositol-3-phosphate and diacylglycerol. PLC ζ is present in the sperm cell acrosome and cytosol but doesn't significantly affect its metabolism. However, after the fusion of sperm and egg membranes, its activity increases as it begins to bind membranes of the egg. It is unknown why PLC ζ is inactive in spermatozoa or any type of somatic cell.

A model describing the activity of PLC ζ in physiological calcium concentrations was developed. In this work, the modeling approach explains the absence of PLC ζ activity in any type of mammalian cells but eggs. It was shown that the presence of phosphoinositide-rich vesicles is required for the PLC ζ activity in mature mammalian eggs.

Keywords: Phospholipase C ζ , Oocyte, Spermatozoa, Calcium signaling

Introduction

During mammalian fertilization, sperm induces a series of calcium oscillations in the egg that induce egg activation and early embryonic development [1]. Phospholipase C (PLC) ζ is a sperm-specific protein capable of causing Ca²⁺ release [2]. PLC ζ is an enzyme that catalyzes the reaction of phosphatidylinositol-4,5-phosphate (PIP₂) hydrolysis into inositol-3-phosphate (IP₃) and diacylglycerol (DAG). It is present in the sperm cell acrosome and cytosol but doesn't significantly affect its metabolism. However, after the fusion of sperm and egg cell, its activity increases as PLC ζ begins to bind membranes of the egg cell and generate IP₃ [3]. PLC ζ can also initiate calcium oscillations in oocytes without the sperm cell if its expression in the oocyte is artificially enabled [2]. Some eggs fertilized by PLC ζ -null sperm can develop, albeit at significantly reduced efficiency, and after a significant time delay [4].

One of the main questions about PLC ζ is the reason for the absence of its activity in sperm cells, as well as in other cell types like Chinese hamster ovary cells (CHO) [5]. Several hypotheses are explaining the cause of exclusive PLC ζ activity in the oocyte (Fig. 1). One of them assumes that there is an unknown cofactor that activates PLC ζ (Fig. 1A). This cofactor is located in oocyte cytosol [3]. The alternative explanation suggests that in sperm PLC ζ is bound to some kind of inhibitor (Fig. 1B), and after fusion with the oocyte, the complex with inhibitor dissolves. This hypothesis partially contradicts data from [5], which demonstrated that when expressed in Chinese hamster ovary cells PLC ζ shows no significant impact on calcium concentration. None of these hypotheses are supported by direct evidence.

The alternative hypothesis is based on PIP₂-rich vesicles in oocytes (Fig. 1C, D). These vesicles have much higher relative surface space than the membrane, and it was demonstrated that a large part of PLC ζ bounds to these vesicles [6], [5].

The modeling approach has been successfully used to study the calcium activation mechanism in both the sperm and egg cells [7], [8], but no work exists on the PLC ζ activity regulation. In this work, we utilize the modeling approach to clarify the mechanisms regulating the PLC ζ activity in sperm and egg cells.

Materials and methods

Computational model construction and integration

To study the PLC ζ activity in mammalian eggs, a computer model was developed. First, the ordinary differential equations were constructed based on laws of chemical kinetics (either mass action law, Henry-Michaelis-Menten kinetics, or Hill functions). Kinetic equation parameters were either taken from the literature or estimated based on existing experimental data. The final model consisted of 18 equations with two unknown parameters. The model was solved using the LSODA method.

Figure 1. Major hypotheses explaining PLC ζ activity in the oocyte. (A) The first hypothesis is that the PLC ζ acquires the ability to cleave the oocyte membrane PIP2 ($PIP2_m$) and produce IP3 upon activation by an unspecified PLC ζ activator A present in oocytes. (B) The second hypothesis is the presence of an unspecified PLC ζ inhibitor in all cell types but eggs (C, D). The third hypothesis suggests that mammalian oocytes contain vesicles higher in PIP2 ($PIP2_v$) content, and PLC ζ is targeted on the vesicle surface.

Results and discussion

Mathematical model of PLC ζ activity

Before checking the hypotheses described above, an accurate mathematical model of PLC ζ activity that can describe the existing experimental data [6],[9] was developed. According to multiple authors ([6],[9]) the unique feature of PLC ζ is its high cooperativity for Ca^{2+} binding, which is performed by its four EF-hand domains. Its Hill coefficient varies from 3.8 to 4.3 ([10], [11],[5],[9]) according to different sources. However, as it was shown below, such a high value for the Hill coefficient is incompatible with the data demonstrated in these articles. Therefore, the Hill coefficient was calculated using two different methods. It was shown that its value, according to the data from [10], is close to 1.1.

A simple mathematical model which could predict calcium dependence of PLC ζ activity in physiologically relevant concentrations of calcium (10^{-8} – 10^{-6} M) was created. It was based on Klotz equation (1), and a hypothesis that every following calcium ion binds with an equilibrium coefficient equal to the previous one multiplied on cooperativity constant α (2-5):

$$B = \frac{[PLC\zeta-Ca] + 2 * [PLC\zeta-2Ca] + 3 * [PLC\zeta-3Ca] + 4 * [PLC\zeta-4Ca]}{PLCtotal} = \frac{Keq * [Ca] + 2 * a * Keq^2 * [Ca]^2 + 3 * a^3 * Keq^3 * [Ca]^3 + 4 * a^6 * Keq^4 * [Ca]^4}{1 + Keq * [Ca] + a * Keq^2 * [Ca]^2 + a^3 * Keq^3 * [Ca]^3 + a^6 * Keq^4 * [Ca]^4} \quad (1)$$

$$K2 = K1 * a \quad (2)$$

$$K3 = K1 * a^2 \quad (3)$$

$$K4 = K1 * a^3 \quad (4)$$

$$Kr = K1/Keq \quad (5)$$

Here **K1** is the constant for the first calcium ion binding, **K2** – for the second calcium ion binding, **K3** – for the third, **K4** – for the fourth, **Keq** is the equilibrium constant for the first calcium ion binding reaction, **a** is cooperativity constant, **[PLC ζ -Ca]** is the concentration of PLC ζ bound with one Ca^{2+} ion in the steady-state, **[PLC ζ -2Ca]** is the concentration of PLC ζ bound with two Ca^{2+} ions in the steady-state, **[PLC ζ -3Ca]** with three, **[PLC ζ -4Ca]** with four. **PLCtotal** is the total concentration of all forms of PLC ζ summarized. **B** is a parameter in the Klotz equation, representing the molar ratio of total PLC ζ and calcium bound with it.

Using equations 2-5, a mathematical model based on mass action law was developed. Calcium concentration in this model was fixed, while variables represent dynamics of PLC ζ bound with different

amount of calcium ions:

$$\frac{d[PLCz]}{dt} = -K1 * [Ca] * [PLCz] + Kr * [PLCz_Ca] \quad (6)$$

$$\frac{d[PLCz_Ca]}{dt} = K1 * [Ca] * [PLCz] - Kr * [PLCz_Ca] - K2 * [Ca] * [PLCz_Ca] + Kr * [PLCz_2Ca] \quad (7)$$

$$\frac{d[PLCz_2Ca]}{dt} = K2 * [Ca] * [PLCz_Ca] - Kr * [PLCz_2Ca] - K3 * [Ca] * [PLCz_2Ca] + Kr * [PLCz_3Ca] \quad (8)$$

$$\frac{d[PLCz_3Ca]}{dt} = K3 * [Ca] * [PLCz_2Ca] - Kr * [PLCz_3Ca] - K4 * [Ca] * [PLCz_3Ca] + Kr * [PLCz_4Ca] \quad (9)$$

$$\frac{d[PLCz_4Ca]}{dt} = K4 * [Ca] * [PLCz_3Ca] - Kr * [PLCz_4Ca] \quad (10)$$

Here, **K1** is constant for the first calcium ion binding, **K2** – for the second, **K3** – for third, **K4** – for fourth, **Keq** is the equilibrium constant for the first calcium ion binding reaction, **a** is cooperativity constant. The concentration of PLCζ, **[PLCz_Ca]** is the concentration of PLCζ bound with one Ca ion in the steady-state, **[PLCz_2Ca]** is the concentration of PLCζ bound with two Ca²⁺ ions in the steady-state, **[PLCz_3Ca]** - with three, **[PLCz_4Ca]** - with four.

We have investigated the steady-state of the model. The details are given in S1.

The model **% of activity** was calculated as follows:

$$\frac{0.03 * [PLCz_2Ca] + 0.22 * [PLCz_3Ca] + [PLCz_4Ca]}{PLCtotal} = \% \text{ of activity} \quad (11)$$

This parameter depends on the concentrations of PLCζ forms associated with two, three or four calcium ions. The coefficients for recalculating the activity of the concentrations of these forms are taken from the data on the maximum activity of PLCζ with the deletion of one or two EF-hands [12]. For PLCζ bound to three calcium ions, the data on PLCζ with one EF-hand deletion was used (in this case, activity was 22% of the native enzyme form activity). For PLCζ bound to two calcium ions, the data on PLCζ with two EF-hand deletion was used (in this case, activity was 3 % of the native enzyme form activity).

Parameter optimization was performed using a genetic algorithm. The experimental enzyme activity in relation to the maximal (**% of activity**) was obtained from the literature data.

The model predicted equilibrium constant **Keq** = **8.3*10⁸ M⁻¹** and cooperativity constant **a** = **0.33**. Using these values, a dependency of PLCζ activity on calcium concentration was built (Fig. 2A).

Hill approximation of PLCζ activity

As an alternative approach to describing PLCζ activity, we used the Hill equation. The Hill equation for an enzyme has the following form:

Figure 2. Validation of the models. (A) Validation of our calcium-binding model with experimental data from [10]. (B) Comparison of Hill equation for n=4.3 with experimental data from [10]. (C) Comparison of Hill equation for n=0.9 with experimental data from [10]. (D) Comparison of Hill equation for n=1.1 with experimental data from [10].

$$\frac{E}{E_{max}} = \frac{[Ca]^n}{[EC50]^n + [Ca]^n} \quad (12)$$

where E is PLC ζ activity, E_{max} is on PLC ζ maximum activity, $EC50$ is calcium concentration required for 50% activity, $[Ca]$ is calcium concentration, n is Hill coefficient.

If we take the Hill coefficient from [11], ($n=4.3$), we will receive calcium dependency, which can be observed on Fig. 2B. It approximates the experimental data with much higher standard deviation than our model described in (6)-(10). The same stands true for the Hill coefficient obtained from [12] ($n=0.9$).

Hill coefficient could be estimated from data on calcium concentration at different activities ($EC90$ and $EC10$ – 90% and 10% of maximum activity accordingly).

$$n = \frac{\log_{10}(91)}{\log_{10}(EC90/EC10)} \quad (13)$$

For PLC ζ activity predicted by our model, $EC90$ is $2.7 \cdot 10^{-7}$ M, while $EC10$ is $5.3 \cdot 10^{-9}$ M. By using equation (13), we can find that Hill coefficient for PLC ζ is equal to 1.12.

The same procedure was repeated for data from [11]. For PLC ζ , $EC90$ is 4.3×10^{-7} M, and $EC10$ is 8×10^{-9} M. By using the same equation (13), we can find that the actual experimental Hill coefficient for PLC ζ is approximately equal to 1.1, which corresponds well with the predictions of our model.

The complete model of phospholipase C activity in mammalian spermatozoa and egg cells

The complete model of PLC ζ activity on egg (Fig. 3A) and sperm membranes (Fig. 3B) was developed.

In the sperm and the egg, two similar reaction schemes were used. PLC ζ could bind calcium and the available plasmatic membranes independently, but only the form that was bound both to the membrane and four calcium ions could produce IP $_3$. IP $_3$ was removed from the system with a constant rate. The differences between two models were as follows: the egg contains vesicles rich in phosphoinositides, while sperm does not. All reactions occurring on the vesicle membrane duplicate the reactions occurring on the plasmalemma. The reaction constants between schemes did not differ, except for the presence of a separate constant for PLC binding to vesicles in the oocyte model and the rate constant for inositol-3-phosphate degradation.

Two models were created using the schemes described above: a model for PLC ζ activity in the oocyte (Fig. 3, A) and sperm cell (Fig. 3, B). The model equations are described in S2. The details of the model parameters are given in Table S1.

The dependence of IP $_3$ concentration on PIP $_2$ concentration on various membranes is shown in Fig. 4. A single steady-state existed in both of the models. The stationary concentration of IP $_3$ in the spermatozoon was approximately 50-60 nM, and in the oocyte, IP $_3$ concentration was about 200 nM (which is above the response threshold of 130 nM [13] for IP $_3$ receptors present both in sperm cells and oocytes [14], [15]). Thus, according to the point model, the activity of PLC ζ alone should be sufficient to initiate calcium oscillations in the oocyte.

In the sperm cell, the final concentration of inositol-3-phosphate, depending on the concentration

Figure 3. Scheme of the full model. (A) Reactions in the oocyte, PLC ζ – calcium-free PLC ζ , PLC ζ_{Ca} – PLC ζ , bound with one calcium ion, PLC ζ_{2Ca} – PLC ζ , bound with two calcium ions, PLC ζ_{3Ca} – with three, PLC ζ_{4Ca} – with four. PLC ζ_m – PLC ζ , bound with the cell membrane, PLC $\zeta_{Ca m}$ – PLC ζ , bound with the cell membrane and one calcium ion, PLC $\zeta_{2Ca m}$ – PLC ζ , bound with the cell membrane and two calcium ions, PLC $\zeta_{3Ca m}$ – with the cell membrane and three calcium ions, PLC $\zeta_{4Ca m}$ – with the cell membrane and four calcium ions. PIP $_2$ and PIP $_2_v$ – are phosphatidylinositol-4,5 – bis-phosphates on cell and vesicle membrane accordingly. DAG and DAG $_v$ – diacylglycerol on cell membrane and vesicles accordingly. IP $_3$ – inositol-3-phosphate. PLC ζ_v – PLC ζ , bound with vesicles, PLC $\zeta_{Ca v}$ – PLC ζ , bound with vesicles and one calcium ion, PLC $\zeta_{2Ca v}$ – PLC ζ , bound with vesicles and two calcium ions, PLC $\zeta_{3Ca v}$ – with vesicles and three calcium ions, PLC $\zeta_{4Ca v}$ – with vesicles and four calcium ions. (B) The sperm cell model is identical to the oocyte model except for the absence of the vesicles.

Figure 4. Results in the whole model. (A) Dependence of the IP3 concentration on sperm membrane PIP2 content. (B) Dependence of the IP3 concentration on PIP2 content in oocyte vesicles.

of PIP2, reaches a plateau long before reaching even half of the threshold concentration (130 nM).

A decrease in the concentration of phosphatidylinositol-4,5-bisphosphate on the surface of vesicles by a factor of 10 would reduce the final concentration of inositol-3-phosphate to approximately 20–30 nM and eliminate the possibility of calcium oscillations in the oocyte solely due to the activity of PLC ζ (Fig. 4B). This explains both the absence of oscillations upon initiation of PLC ζ expression in somatic cells [2] and the absence of oscillations under similar conditions at the early stages of oocyte maturation, before the formation of phosphatidylinositol-4,5-bis-phosphate-rich vesicles [16].

Conclusions

Here we have built a mathematical model of PLC ζ activity in mammalian egg and sperm cells and validated it on existing experimental data. It was theoretically shown that the activity of PLC ζ in the sperm is insufficient for the synthesis of IP3 in sufficient quantities to initiate calcium oscillations. Its activity on vesicles in egg cells creates a total concentration of IP3 close to 200 nM, which is above the border value for initiation of Ca oscillations.

The results obtained support the hypothesis of the phosphoinositide composition of egg vesicles as the main factor in the effectiveness of PLC ζ as an initiator of calcium oscillations. They exclude the possibility of calcium oscillations in the sperm cell, which is consistent with all currently available experimental data.

Author Contributions

The whole work was completed by A.K.T.

Acknowledgements

Author is grateful to Miss Julia Jessica Korobkina and Dr. Anastasia Svesnikova (CTP PCP RAS, Moscow, Russia) for valuable discussions.

References

1. Saleh A, Kashir J, Thanassoulas A, Safieh-Garabedian B, Lai FA, Nomikos M. Essential Role of Sperm-Specific PLC-Zeta in Egg Activation and Male Factor Infertility: An Update. *Frontiers in Cell and Developmental Biology* 2020;8. <https://doi.org/10.3389/fcell.2020.00028>.
2. Yoshida N, Amanai M, Fukui T, Kajikawa E, Brahmajosyula M, Iwahori A, et al. Broad, ectopic expression of the sperm protein PLCZ1 induces parthenogenesis and ovarian tumours in mice. *Development* 2007;134:3941–52. <https://doi.org/10.1242/dev.007930>.
3. Kashir J, Nomikos M, Lai FA. Phospholipase C zeta and calcium oscillations at fertilisation: The evidence, applications, and further questions. *Advances in Biological Regulation* 2018;67:148–62. <https://doi.org/10.1016/j.jbior.2017.10.012>.
4. Hachem A, Godwin J, Ruas M, Lee HC, Buitrago MF, Ardestani G, et al. PLC ζ is the physiological trigger of the Ca²⁺ oscillations that induce embryogenesis in mammals but offspring can be conceived in its absence. *Development* 2017. <https://doi.org/10.1242/dev.150227>.
5. Phillips S V., Yu Y, Rossbach A, Nomikos M, Vassilakopoulou V, Livanou E, et al. Divergent effect of mammalian PLC ζ in generating Ca²⁺ oscillations in somatic cells compared with eggs. *Biochemical Journal* 2011;438:545–53. <https://doi.org/10.1042/BJ20101581>.
6. Nomikos M, Swann K, Lai FA. Starting a new life: Sperm PLC-zeta mobilizes the Ca²⁺ signal that induces egg activation and embryo development. *BioEssays* 2012;34:126–34. <https://doi.org/10.1002/bies.201100127>.
7. Atri A, Amundson J, Clapham D, Sneyd J. A single-pool model for intracellular calcium

- oscillations and waves in the *Xenopus laevis* oocyte. *Biophysical Journal* 1993;65:1727–39. [https://doi.org/10.1016/S0006-3495\(93\)81191-3](https://doi.org/10.1016/S0006-3495(93)81191-3).
8. Simons J, Fauci L. A Model for the Acrosome Reaction in Mammalian Sperm. *Bulletin of Mathematical Biology* 2018;80:2481–501. <https://doi.org/10.1007/s11538-018-0478-3>.
 9. Nomikos M, Theodoridou M, Elgmati K, Parthimos D, Calver BL, Buntwal L, et al. Human PLC exhibits superior fertilization potency over mouse PLC in triggering the Ca^{2+} oscillations required for mammalian oocyte activation. *Molecular Human Reproduction* 2014;20:489–98. <https://doi.org/10.1093/molehr/gau011>.
 10. Kouchi Z, Fukami K, Shikano T, Oda S, Nakamura Y, Takenawa T, et al. Recombinant Phospholipase C ζ Has High Ca^{2+} Sensitivity and Induces Ca^{2+} Oscillations in Mouse Eggs. *Journal of Biological Chemistry* 2004;279:10408–12. <https://doi.org/10.1074/jbc.M313801200>.
 11. Nomikos M, Blayney LM, Larman MG, Campbell K, Rossbach A, Saunders CM, et al. Role of Phospholipase C- ζ Domains in Ca^{2+} -dependent Phosphatidylinositol 4,5-Bisphosphate Hydrolysis and Cytoplasmic Ca^{2+} Oscillations. *Journal of Biological Chemistry* 2005;280:31011–8. <https://doi.org/10.1074/jbc.M500629200>.
 12. Kouchi Z, Shikano T, Nakamura Y, Shirakawa H, Fukami K, Miyazaki S. The Role of EF-hand Domains and C2 Domain in Regulation of Enzymatic Activity of Phospholipase C ζ . *Journal of Biological Chemistry* 2005;280:21015–21. <https://doi.org/10.1074/jbc.M412123200>.
 13. De Young GW, Keizer J. A single-pool inositol 1,4,5-trisphosphate-receptor-based model for agonist-stimulated oscillations in Ca^{2+} concentration. *Proceedings of the National Academy of Sciences* 1992;89:9895–9. <https://doi.org/10.1073/pnas.89.20.9895>.
 14. Kuroda Y, Kaneko S, Yoshimura Y, Nozawa S, Mikoshiba K. Are there inositol 1,4,5-trisphosphate (IP3) receptors in human sperm? *Life Sciences* 1999;65:135–43. [https://doi.org/10.1016/S0024-3205\(99\)00230-1](https://doi.org/10.1016/S0024-3205(99)00230-1).
 15. Cai X, Li X, Qi H, Wei F, Chen J, Shuai J. Comparison of gating dynamics of different IP₃ R channels with immune algorithm searching for channel parameter distributions. *Physical Biology* 2016;13:056005. <https://doi.org/10.1088/1478-3975/13/5/056005>.
 16. Parrington J, Swann K, Shevchenko VI, Sesay AK, Lai FA. Calcium oscillations in mammalian eggs triggered by a soluble sperm protein. *Nature* 1996;379:364–8. <https://doi.org/10.1038/379364a0>.