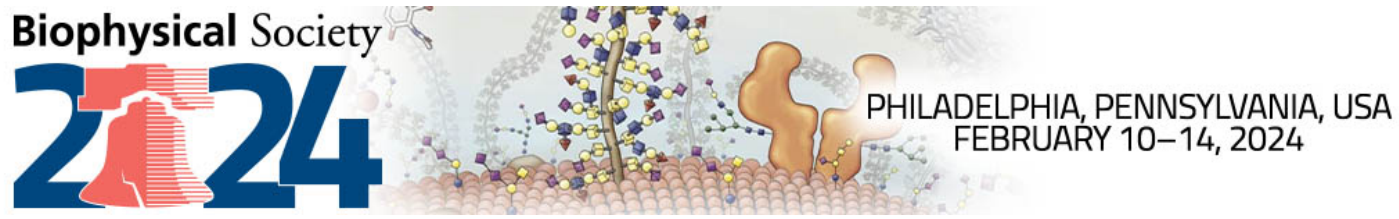


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**ZETA POTENTIAL AS A METRIC FOR MEASURING THE ACTIVITY OF A MEMBRANE-ACTIVE ENZYME**

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**Abstract:**
Phospholipase D (PLD) is an interfacial enzyme that catalyzes the hydrolysis of the ester bond in phosphadylcholine (PC) lipid, producing choline and phosphatidic acid (PA), an important signaling molecule. Thus, this enzyme plays an essential role in cell signaling, membrane lipid metabolism, and membrane remodeling. Activity of PLD is traditionally assessed by assay kits that quantify the amount of choline produced. We previously reported the use of channel conductance as a metric for PLD activity on planar lipid bilayers. Here, we explore the use of membrane zeta potential as a simple and reliable metric for PLD activity on liposomal membranes. Considering that PLD-mediated hydrolysis of zwitterionic PC lipid results in production of anionic PA in the membrane, we hypothesize that membrane zeta potential can serve as a viable parameter to quantify the enzymatic activity of PLD. To test this hypothesis, zeta potential of PC liposomes was measured in the presence of PLD at different time points. Results showed that zeta potential in liposomes changed with time in the presence of PLD and that these time-dependent changes differed with varying concentrations of PLD. The zeta measurements were then used to calculate the amount of PA produced over time based on a calibration curve of zeta potential as a function of PA percentage in liposomes. To confirm the association of zeta potential changes to PLD activity, heat denaturation tests were employed and liposomes incubated with heat-denatured PLD exhibited virtually no change in zeta potential. The results of these experiments were compared to those from commercially available choline assays, showing good agreement. Findings of this work demonstrate that zeta potential can be effectively used as a simple, label-free method for assessing the activity of enzyme-mediated charge modifications on lipid membranes.

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