Supporting Information

Gramicidin Pores Report the Activity of Membrane-Active Enzymes

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Conductivity through the Bulk Membrane of Planar Lipid Bilayers Containing PA Lipid but no gA

Hovis and co-workers have investigated the effect of ionic strength on the organization and topology of supported lipid bilayers composed of PC and PA lipids.[1] These authors demonstrated that lowering the ionic strength led to formation of PA-enriched domains in supported bilayers that contained more than 10 mol% PA. To examine the effect of such organizational change on the permeability of the planar lipid bilayers employed in this work, we probed the conductivity through PC bilayers that contained 0-30mol % of PA lipids under the same condition as the enzymatic assay (in a recording buffer containing 10 mM CsCl, 0.5 mM $CaCl_2$, and 10 mM cesium acetate at pH 5.5) but in

the *absence* of gramicidin pores. Figure S1 illustrates the mean conductance through the examined lipid bilayers with different PA contents but in the absence of gA. These results revealed small variations in conductance of these lipid bilayers (0.3-0.4 pS), however, we did not observe a trend of the change in conductance as a function of X_{PA}. As explained before, these small variations in conductance of ions through the bilayer membrane itself, did not affect the accuracy of the determination of the single channel conductance of gA pores since we based the analysis exclusively on step-changes in current which resulted from opening and closing of individual gA pores.

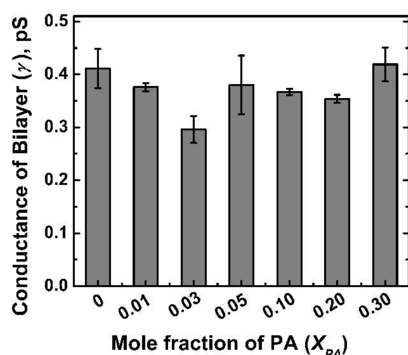

Figure S1. Effect of PA content in planar lipid bilayers on the conductivity through the bilayer in the *absence* of gA. Error bars represent standard error of the mean ($N \geq 3$).

Lag Phase in PLD Hydrolysis

Figure S2, which shows a plot of X_{PA} as a function of time, illustrates a clear biphasic behavior with two distinct linear slopes. In the first phase, defined as lag phase (the time interval from addition of PLD until X_{PA} reached a value of ~0.03), which started immediately after addition of PLD and typically continued for ~5-9 min, X_{PA} increased slowly with time in a linear fashion. Once the mole fraction of PA reached a value of ~0.03, the time-dependent increase of X_{PA} entered a second phase of linear change with a steeper positive slope. In the analysis performed here, we employed the linear slope of the

second phase as the initial hydrolysis rate of PLD (Fig. 7a). The hydrolysis rate after the lag phase was typically 3-6 fold higher than the hydrolysis rate during the lag phase.

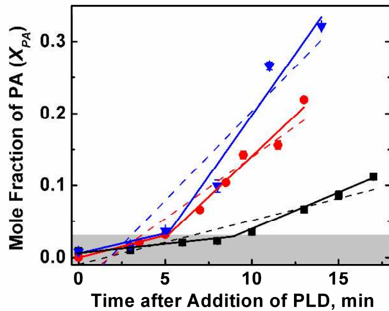

Figure S2. Biphasic change in mole fraction of PA, X_{PA}, in PC membranes after addition of (■) 2.3 (●) 4.2, and (▼) 5.2 units·mL^{-1} PLD. The graph shows the mean value of X_{PA} in planar lipid bilayers after addition of PLD. Error bars represent the standard error of the mean ($N \geq 3$). Dashed lines represent the best linear fits to the data (black: $X_{PA} = -0.009 + 0.006$ (min^{-1}) $\times\ t$ (min), with $N = 8$, $R^2 = 0.86$, red: $X_{PA} = -0.031 + 0.017$ (min^{-1}) $\times\ t$ (min), with $N = 8$, $R^2 = 0.91$, blue: $X_{PA} = -0.045 + 0.025$ (min^{-1}) $\times\ t$ (min), with $N = 6$, $R^2 = 0.87$). The first part of the solid lines represents the best linear fits to the points in the lag phase (black: $X_{PA} = 0.006 + 0.003$ (min^{-1}) $\times\ t$ (min), with $N = 4$, $R^2 = 0.85$, red: $X_{PA} = 0 + 0.006$ (min^{-1}) $\times\ t$ (min), with $N = 3$, $R^2 = 1$, blue: $X_{PA} = 0.005 + 0.006$ (min^{-1}) $\times\ t$ (min), with $N = 3$, $R^2 = 0.78$) and the second part of the solid lines represent the best linear fits to the points after the lag phase (black: $X_{PA} = -0.061 + 0.009$ (min^{-1}) $\times\ t$ (min), with $N = 4$, $R^2 = 0.99$, red: $X_{PA} = -0.086 + 0.022$ (min^{-1}) $\times\ t$ (min), with $N = 6$, $R^2 = 0.96$, blue: $X_{PA} = -0.142 + 0.034$ (min^{-1}) $\times\ t$ (min), with $N = 4$, $R^2 = 0.93$). The gray area illustrates the lag phase.

Signal Amplification through Gramicidin A Pores

Ion channels are attractive for sensing applications due to their inherent amplification capability:[2-4] Opening of a single ion channel can lead to the flux of 10^3-10^6 ions per millisecond through the channel.[4] Previous studies have employed gA pores[5] to sense the electrical charge on the

lipids surrounding the channel in the membrane.[2,4] These studies showed that a slight difference in the electrical charge on the lipid membrane can lead to an amplified change in single channel conductance of gA. The present assay took advantage of this amplifying effect of gA pores to detect the enzymatic activity of PLD and PLC. For instance, Fig. 6a reveals that an increase of only 5% in the mole fraction of negatively-charged PA lipids in a PC membrane (i.e., a change from $X_{PA} = 0$ to $X_{PA} = 0.05$) led to an increase in single channel conductance of gA pores from $\gamma = 3.5$ pS to $\gamma = 6.5$ pS, corresponding to an increase of 86%. In this assay, therefore, gA pores offered an amplification factor of ~17 (0.86 / 0.05) for sensing the enzymatic activity of PLD.

Effect of Choline Chloride on the Single Channel Conductance of Gramicidin A Pores

Phospholipase D catalyzes the hydrolysis of phosphatidylcholine (PC), producing soluble choline and phosphatidic acid (PA) lipid.[6] In order to examine the effect of the soluble product of this reaction, choline, on the conductance of gA we formed planar lipid bilayers of DiPhyPC and monitored the conductance of gA in the presence of choline chloride (Fluka).

During the experiments with PLD, the mole fraction of the enzymatic product PA typically reached a final value of ~0.3 which corresponded to ~45 nanomoles of PA lipids and an average concentration of ~15 μM in a 3 mL compartment. We, therefore, estimated the concentration of the soluble product of this enzymatic reaction, choline, to be ≤ 15 μM and examined the effect of choline chloride on the conductance of gA within a final concentration range of 0-20 μM. We carried out these single channel recordings in an electrolyte solution containing 10 mM CsCl, 10 mM cesium acetate, pH 5.5 and added choline chloride from a stock solution in the same electrolyte. These experiments revealed that the presence of 0-20 μM choline chloride did not significantly affect the single channel conductance of gA (change < 3%).

Limit of Detection of the Assay

The limit of detection (LOD) of the PLD assay and the PLC assay presented here, according to the definition of LOD = 3 × standard deviation (SD), is equal to a mole fraction of PA, X_{PA}, of 0.003 and to a mole fraction of PI, X_{PI}, of 0.078 respectively. We calculated the LOD for the PLD enzymatic assay considering a SD of 0.05 pS for the conductance of gA in a PC bilayer. For this analysis, we employed Eq. 1 (in the main text) and standard error propagation rules to convert the smallest detectable change in the conductance of gA, γ, to the smallest detectable change in X_{PA} in the membrane as the detection limit of this assay. A similar approach for the PLC enzymatic assay, considering a SD of 1.45 pS for the conductance of gA in a bilayer composed of PC and 10 mol% PI and employing Eq. 4 gave a value of 0.078 as the smallest detectable change in X_{PI} in the membrane as the detection limit of this assay.

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