New and Notable

A New Model of Interfacial Kinetics for Phospholipases

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Lipid bilayers are amphipathic structures consisting of a central hydrocarbon core and two flanking polar interfacial regions, which are dynamic and may contain >1000 different types of lipid. Heterogeneous catalysis can occur on the water-lipid interface of membrane bilayers where lipid metabolism is necessary to regulate processes such as signal transduction, cell growth and division, and membrane trafficking. Phospholipases are a class of interfacial enzymes that catalyze hydrolysis of ester bonds in phospholipids where their activity depends upon the membrane structure and interfacial properties (1–4). Phospholipases play key roles in lipid metabolism, cell signaling, and meiosis (5,6), among other processes. Enzymatic reactions at the water-lipid interface are unlike those enzymatic reactions that occur in solutions where kinetic behavior at the lipid-water interface cannot usually be described with simple Michaelis-Menten kinetic analysis.

To describe catalytic reactions by interfacial enzymes, a number of kinetic models have been developed over the last few decades to best interpret how interfacial enzymes regulate these reactions. Verger et al. (7) set an early precedent by proposing the first kinetic model, which combined the Michaelis-Menten model with the interfacial activation of enzymes. This led to further development of kinetic analysis and models of interfacial catalysis on lipid vesicles, micelles, and lipid monolayers. This includes the "surface dilution kinetics" on micelles (8) and the "scooting and hopping models of enzyme action" (9). These models have been limited, however, as they assume enzymatic products are soluble in an aqueous environment and also do not account for product accumulation of lipids with long acyl chains that remain in the membrane bilayer. For instance, phospholipase D (PLD), an important enzyme in signaling and cell migration (10), catalyzes the production of the glycerophospholipid phosphatidic acid (PA), which has long acyl chains and remains in the membrane bilayer. To date, micelles have been used in most studies of interfacial catalysis and have applied the surface dilution model to interpret the kinetics of PLD, which has limited the interpretation of PLD catalysis on membrane bilayers.

In this issue of *Biophysical Journal*, a new model for the interfacial kinetics of PLD has been proposed by Majd et al. (11), which accounts for the accumulation of products, namely PA in PLD activity. To the best of my knowledge, this work presents the first quantitative kinetic analysis of PLD at the membrane interface. Previous work by Majd et al. (12) had shown that planar lipid bilayers with chemical and electrical access to both sides of the lipid membrane could be used to monitor catalytic reactions at the lipid-water interface. Importantly, Majd et al. provide a model that accounts for the interaction between PLD and its reaction product PA. This method, which was previously used to demonstrate PLD and phospholipase C activities, is based on lipase-induced changes in the electrical charge of lipid bilayers and on the associated change in ion concentration near the lipid interface through pores of the ion channel-forming peptide gramicidin A.

The work of Majd et al. (11) extends the basic kinetic model for interfacial catalysis where their analysis now accounts for product activation and substrate depletion. This new model better describes kinetic behavior as the specificity constant, which is a measure of catalytic efficiency of the enzyme. The interfacial quality constant is also defined, which accounts for the physicochemical properties of the interface including the charge density, orientation and conformation of molecules, water structure, and role of viscosity on enzyme processing. Both the specificity constant and the interfacial quality constant agree with values previously reported for phospholipase A on monolayers (13).

For investigation of PLD interfacial kinetics, the authors began with the general scheme proposed by Verger et al. (7) where there is reversible desorption of the soluble enzyme (E) to an interfacial form (E*). This model then exhibits a two-dimensional Michaelis-Menten reaction where the phospholipase locates its substrate (S*) and forms the Michaelis complex (E*S*), which is followed by catalysis to products that are either released (P) or may remain in the membrane (P*). This model has some shortcomings when it comes to an enzyme like PLD, as the model assumes the product quickly diffuses away and does not affect succeeding reactions. The authors find that as PA accumulates, PLD activity is increased—which likely affects the equilibrium of the soluble (E) and interfacial enzyme (E*). To account for this change in the reaction, PLD adsorption-desorption was modeled as a bimolecular association process. Furthermore, the authors account for substrate depletion and dilution in the bilayer as PA accumulates. For a full derivation of the appropriate equations to fit these parameters, I refer you to the article by Majd et al. (11).

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To quantify PLD activity using the aforementioned assay system, PLD was added to both sides of the bilayer, which led to a time-dependent increase in channel conductance. Majd et al. then made a calibration curve of the conductance as a function of PA concentration in the bilayers. They found that PLD activity on the phosphatidylcholine bilayer begins with a lag phase but, as PA accrues in the membranes, the activity of PLD accelerates. This is likely attributed to PA domains that form as PLD acts on PC, which increase the binding of PLD to the membrane and the rate of activity (12). As PA further accumulates in the bilayer, this leads to substrate depletion and lowers the rate of PLD hydrolysis. The kinetic model derived in this article accurately describes the kinetic behavior of PLD as it accounts for the effects of PA accumulation and substrate depletion.

This methodology and new interpretation of kinetics is applicable to other phospholipases and perhaps interfacial enzymes such as lipid kinases and phosphatases. As with most techniques, there are some potential limitations as well. For instance, the authors' setup is planar in nature, and is likely a great model for enzyme binding and activity. Recently, however, membrane curvature has been shown to also play a role in some enzymes' activities. Moreover, product appearance of PA by PLD catalysis could alter membrane shape as PA has a small headgroup and two acyl chains, which can result in negative curvature changes in membranes. Additionally, some phospholipases may themselves induce membrane curvature changes through interfacial catalysis or membrane binding. That being said, this new platform still exceeds prior methods such as micelles and monolayers for accurately monitoring the kinetics of interfacial catalysis of PLD and potentially other phospholipases. Taking into account product formation and its interaction with the enzyme at the interface should greatly shape our understanding of interfacial kinetics for a multitude of enzymes involved in regulation of lipid signaling, membrane trafficking, and bilayer structure.

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REFERENCES

1. Panaiotov, I., and R. Verger. 2000. Enzymatic reactions at interfaces: interfacial and temporal organization of enzymatic lipolysis. *In* Physical Chemistry of Biological Interfaces. A. Baszkin and W. Norde, editors. Marcel Dekker, New York, pp. 359–400.

2. Deems, R. A., B. R. Eaton, and E. A. Dennis. 1975. Kinetic analysis of phospholipase A_2 activity toward mixed micelles and its implications for the study of lipolytic enzymes. *J. Biol. Chem.* 250:9013–9020.

3. Leidy, C., J. Ocampo, …, G. H. Peters. 2011. Membrane restructuring by phospholipase A_2 is regulated by the presence of lipid domains. *Biophys. J.* 101:90–99.

4. Gudmand, M., S. Rocha, …, T. Heimburg. 2010. Influence of lipid heterogeneity and phase behavior on phospholipase A_2 action at the single molecule level. *Biophys. J.* 98:1873–1882.

5. Vines, C. M. 2012. Phospholipase C. *Adv. Exp. Med. Biol.* 740:235–254.

6. Leslie, C. C., T. A. Gangelhoff, and M. H. Gelb. 2010. Localization and function of cytosolic phospholipase A2alpha at the Golgi. *Biochimie.* 92:620–626.

7. Verger, R., M. C. E. Mieras, and G. H. de Haas. 1973. Action of phospholipase A at interfaces. *J. Biol. Chem.* 248:4023–4034.

8. Carman, G. M., R. A. Deems, and E. A. Dennis. 1995. Lipid signaling enzymes and surface dilution kinetics. *J. Biol. Chem.* 270:18711–18714.

9. Jain, M. K., and O. G. Berg. 1989. The kinetics of interfacial catalysis by phospholipase A_2 and regulation of interfacial activation: hopping versus scooting. *Biochim. Biophys. Acta.* 1002:127–156.

10. Selvy, P. E., R. R. Lavieri, …, H. A. Brown. 2011. Phospholipase D: enzymology, functionality, and chemical modulation. *Chem. Rev.* 111:6064–6119.

11. Majd, S., E. C. Yusko, …, M. Mayer. 2013. A model for the interfacial kinetics of phospholipase D activity on long-chain lipids. *Biophys. J.* 105:146–153.

12. Majd, S., E. C. Yusko, …, M. Mayer. 2009. Gramicidin pores report the activity of membrane-active enzymes. *J. Am. Chem. Soc.* 131:16119–16126.

13. Panaiotov, I., M. Ivanova, and R. Verger. 1997. Interfacial and temporal organization of enzymatic lipolysis. *Curr. Opin. Colloid Interface Sci.* 2:517–525.