

High Resolution, Real Time Studies of Protein-Lipid Interactions Using Hemilayer Membrane Mimics

Introduction

Dual Polarisation Interferometry (DPI) is an important enabling tool for cell biology, particularly the study of membrane proteins, their behaviour and interactions ⁽¹⁾. This application note describes the use of DPI for the real time, quantitative analysis of protein-lipid interactions implicated in the formation of *lamellipodia* structures involved in cell migration. The molecular system under investigation involved phospholipids commonly found in cell membranes and known to be involved in the regulation of a range of membrane proteins, PtdIns(4,5)P₂ (D-myo-phosphatidylinositol 4,5-bisphosphate known as **PIP2**) and PtdIns(3,4,5)P₃ (D-myo-phosphatidylinositol 3,4,5-triphosphate known as **PIP3**) and their interactions with WAVE2, a WASP family verprolin homologous protein.

Cell migration is one of the ways that cells respond to signals from the extracellular environment. Receptor molecules on the plasma membrane sense the location and intensity of extracellular signals and determine the appropriate direction for cell movement. Recent studies have revealed the important roles played by membrane lipids and the cytoskeleton in cell migration. It is clear that cells recruit the PIP3 at the leading edge to establish cell polarity in response to chemo-attractant gradients. However, the mechanism of how this polarity is followed by formation of the leading edge is still obscure. The protein WAVE2 is expressed ubiquitously in various tissues, and several researchers have reported that localization of WAVE2 at the leading edge is crucial for *lamellipodium* formation. However, the molecular mechanisms underlying this localization are poorly understood.

In this study, a membrane mimic formed by self-assembly of solubilised phospholipid deposited on a C18 surface was used to support the PIP molecules under investigation. Subsequently, we investigated the affinity of interaction between the protein WAVE2 and both PIP2 and PIP3 supported in this phospholipid hemilayer, in an attempt to confirm which PIP binds preferentially to WAVE2.

Experimental

The DPI experiments were performed on a Farfield **AnaLight**[®] instrument. The surface used was a C-18 functionalised silicon oxynitride **AnaChip**[™]. The temperature of the samples was controlled throughout to 20°C. All buffers and reagents were analytical grade or higher, and solutions were degassed prior to use.

Lipid Hemilayer Membrane Mimic Formation: The phospholipid vesicles used in these experiments contained PC:PtdIns:PIP2 or PIP3 at 48:48:4 (mole ratio) and were prepared by extrusion through a 0.1µm cyclopore filter. PIP2 and PIP3 vesicles were coated on the surface of different channels of the C-18 **AnaChip**[™]. The lipids were bound in a monolayer onto C-18 tips, forming the lipid bilayer-like structure composed of C-18 carbon chains and lipids. The buffer used was 10mM HEPES, 150mM NaCl, 3mM EDTA, pH7.5.

WAVE2 Protein – PIP Lipid Interaction Studies: Studies of the interactions between WAVE2 and PIP2 and PIP3 were performed by immobilizing vesicles containing PIP2 and PIP3 on different instrument channels as above, and introducing WAVE2 to both simultaneously. It is known that the pleckstrin homology (PH) domain of phospholipaseCδ1 (PLCδ1) shows a strong affinity for PIP2, and the PH domain of Akt interacted specifically with PIP2 and PIP3, and therefore these were included as controls. All proteins were injected at different concentrations in the same buffer (association phase). The **AnaChip**[™] was regenerated by injection of 10mM NaOH. Bindings were examined at four or more different protein concentrations, and then K_D, k_{ass}, and k_{diss} values were calculated from curve fitting over the initial 60 seconds of association.

Results and Discussion

WAVE2 Protein – PIP Lipid Interaction Studies: **Figure 1** illustrates the binding curves for the proteins on the respective instrument channels containing PIP2 and PIP3. The corresponding k_{ass}, k_{diss} and K_D values are tabulated in **Figure 2**. The K_D was calculated from curve fitting and the errors for each point from the fitted curve were within ±

0.01% and $\pm 0.03\%$, respectively. It is concluded that WAVE2 binds preferentially to PIP3 rather than to PIP2 *in vitro*, supporting the view that recruitment of WAVE2 by PIP3 is an essential process for *lamellipodia* formation at the leading edge.

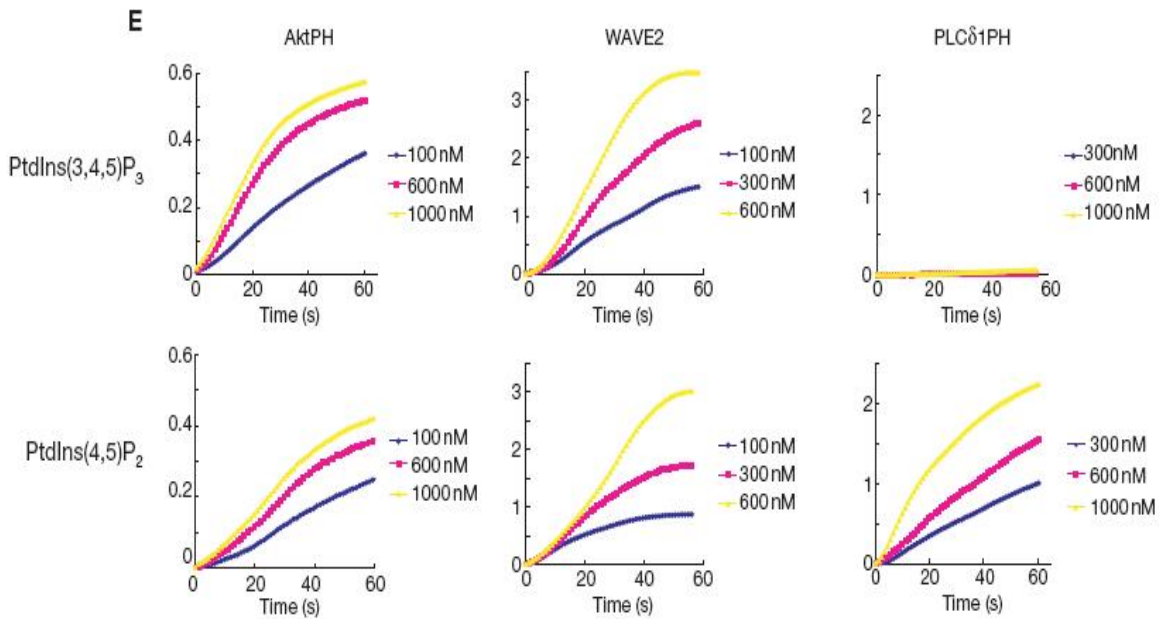


Figure 1: Association curves for PIP3 (top) and PIP2 (bottom) with WAVE2, also showing positive (AktPH) and negative (PLCδ1PH) control

		PIP2 vesicle	PIP3 vesicle
AktPH	k _{ass}	1.3×10^{-5}	4.5×10^{-5}
	k _{diss}	3.1×10^{-2}	1.1×10^{-2}
	K _D	2403 nM	246 nM
	error	0.00%	0.30%
WAVE2	k _{ass}	5.1×10^{-5}	8.3×10^{-5}
	k _{diss}	6.3×10^{-2}	1.5×10^{-2}
	K _D	1220 nM	185 nM
	error	0.03%	0.01%
PLCδ1PH	k _{ass}	3.1×10^{-5}	
	k _{diss}	6.7×10^{-3}	
	K _D	213 nM	ND*
	error	0.01%	

Figure 2 k_{ass}, k_{diss} and K_D values calculated from the binding curves showing WAVE2 has a much higher affinity for PIP3 vesicles than for PIP2 vesicles

(*At these concentrations PLCd1PH binding to PIP3 is so weak affinity constants were not calculated)

Conclusions and Benefits

These studies demonstrate DPI as an enabling technique for the formation of phospholipid membrane mimics and the subsequent study of the interactions of proteins with their phospholipid-binding partners. A simple self-assembled hemilayer can be formed by deposition of phospholipids in liposome buffer or by the rupture of vesicles on a C-18 surface. Kinetics of interaction can be determined directly from the binding responses.

These experiments show DPI can be applied to the study of membrane protein systems. The **AnaLight**[®] instruments and their experimental protocols give the researcher a unique combination of high-resolution data in real time on thickness, refractive index (density) and surface coverage from a bench top technique. The **AnaLight**[®] is an important enabling tool for protein biochemists giving them the ability to:

- Clearly understanding the molecular mechanisms involved in protein-lipid interactions
- Produce and validate viable phospholipid membrane mimics for use in membrane protein studies
- Obtain real time assurance of supporting lipid integrity in such studies
- Generate high-quality affinity parameter data for macromolecular interactions to help in the understanding of the molecular mechanisms underlying cellular processes
- Avoid the limitations and ambiguities that are inherent in other established techniques for such studies, and provide the final results and analysis rapidly

References

⁽¹⁾ Tsukasa Oikawa, Hideki Yamaguchi, Toshiki Itoh, Masayoshi Kato, Takeshi Ijuin, Daisuke Yamazaki, Shiro Suetsugu and Tadaomi Takenawa; **PtdIns(3,4,5)P3 binding is necessary for WAVE2-induced formation of lamellipodia**, *Nature Cell Biology*, 6, 420-428 (2004)

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