Lysophosphatidic acid (LPA)-antibody (504B3) validation by free-solution assay and interferometry identifies off-target binding

Manisha Ray[a], Yasuyuki Kihara[a], Darryl J. Bornhop[b] and Jerold Chun[a*]

[a]Degenerative Disease Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, 92037, USA

[b]Department of Chemistry and Vanderbilt Institute for Chemical Biology, Nashville, TN, 37235, USA

Author emails: Manisha Ray <mray@sbpdiscovery.org>; Yasuyuki Kihara <ykihara@sbpdiscovery.org>; Bornhop, Darryl J <darryl.bornhop@Vanderbilt.Edu>; Jerold Chun <jchun@sbpdiscovery.org>

*Correspondence to: Jerold Chun, MD, PhD. jchun@SBPdiscovery.org

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Abstract (100 words)

Antibody specificity and sensitivity is required in basic and clinical research for ensuring scientific rigor and reproducibility, while off-target cross-reactivity could generate erroneous conclusions. Lysophosphatidic acid (LPA) is a bioactive lipid being targeted clinically by antibody strategies. Here, we reexamined binding properties of a commercially available monoclonal antibody (504B3) reported as specific for LPA using a free-solution assay measured in a compensated interferometric reader. The antibody showed comparable binding affinities to LPA and non-LPA lipids including phosphatidic acid (PA) and lysophosphatidylcholine (LPC). These results may alter conclusions drawn from current and past basic and clinical studies employing anti-LPA antibodies.

Key words: Lysophosphatidic acid, LPA, compensated interferometric reader, ligand binding, antibody, Lpathomab, lysophospholipid

Abbreviations

FSA: Free solution assay; CIR: Compensated Interferometric Reader; ELISA: Enzyme-linked immunosorbent Assay; LPA_1: Lysophosphatidic Acid Receptor 1; LPA: Lysophosphatidic Acid; LPC: lysophosphatidylcholine; PA: Phosphatidic Acid; RI: Refractive Index

Main Text

LPA is a potent, bioactive lipid that acts through six cognate G protein-coupled receptors (LPA_{1-6}) [1, 2]. It is involved in many physiological processes such as cell proliferation, chemotaxis, and smooth muscle contraction, as well as cell survival [3]. Elevated LPA levels are associated with several disease pathologies, including cancer, hydrocephalus, and fibrosis [3-6], implicating therapeutic potential of modulating LPA pathways.

Lpath Inc. (merged with Apollo Endosurgery, Inc. in 2016) developed humanized monoclonal anti-LPA antibodies, including a phase 1a molecule, Lpathomab/LT3015 [7, 8]. The binding affinity and selectivity of Lpathomab/LT3015 was determined by enzyme-linked immunosorbent assay (ELISA) using unnatural, biotinylated lipid species [9], which showed nearly identical, nanomolar affinities to several LPA forms (14:0-, 18:0-, 18:1-, and 18:2-LPAs), without reported binding to other lipid species, including sphingosine 1-phosphate (S1P), 18:0-lysophosphatidylcholine (LPC), phosphatidic acid (PA), phosphatidylcholine (PC), and platelet-activating factor (PAF) [9]. A related anti-LPA antibody, 504B3, whose complementarity determining regions showed 79% identity to the Lpathomab/LT3015 [10], is commercially available (Echelon Biosciences, Product Number: Z-P200) and has been used for preclinical

studies of spinal cord injury [11] and traumatic brain injury [12]. ELISA studies reported similar specificity and selectivity of 5043B compared to Lpathomab/LT3015 [11].

ELISA is a commonly used assay for determining binding affinity, but possesses a number of technical drawbacks that may impact accuracy. These include multiple washing steps that may under or overestimate affinities, immobilization that can increase non-specific interactions through conformational inflexibility [13, 14], and particularly use of biotinylated lipids that limit conformational flexibility of the lipids and introduce non-native epitopes [9]. By comparison, interferometric assays use unmodified, native binding partners such as native amino acids (serotonin, histamine, and dopamine) to their-specific antibodies [15], in free-solution and without need for labels on binding partners. FSAs use refractive index (RI) matched binding *sample* and *reference* solutions that when measured in a CIR, allow for determination of specific binding in real-time. The CIR detects the RI change that occurs as a result of binding-induced conformational and/or hydration changes produced by binding events in the *sample* compared to the null in the non-binding *reference*. The utility of FSA-CIR was recently demonstrated for high-sensitivity detection of native equilibrium binding K_{D}s between LPAs and one of its receptors, LPA_1 [16, 17].

FSA-CIR was used to reevaluate the equilibrium binding affinity (K_D) of the anti-LPA mAb (504B3) against five phospholipids (18:1 LPA, 16:0 LPA, 18:1 LPC, 18:1 S1P, and 18:1-18:1 PA). The *sample* and *reference* were prepared by mixing ligand dilution series (0-100 nM; varied by ligand) with an equivalent volume of the 504B3 solution (*sample*; 10 µg/ml dissolved in PBS, pH 7.4) or PBS only (*reference*)(**Figure 1a**). The FSA signal (ΔRI; refractive index difference between *sample* and *reference*) as measured by the interferometer was plotted against ligand concentrations and showed nanomolar binding affinities of 504B3 to not only 18:1 LPA

($K_D \approx 3.73 \pm 2.8$ nM), but also to 18:1 LPC ($K_D \approx 8.5 \pm 2.6$ nM) and PA ($K_D \approx 3.3 \pm 2.7$ nM), with weaker affinity for 16:0 LPA **(Figure 1b; Table SI:1)**. Endogenous plasma concentrations of LPC (100-300 μm) range beyond 100X LPA concentrations (0.1-2 μm) [18-20], indicating that a vast majority of 504B3 and related antibodies used *in vivo* would be bound to LPC and PA rather than LPA. These results raise concerns about the use of this and related anti-LPA antibodies in basic and clinical research, such as in reports on protective effects in spinal cord injury [11] and traumatic brain injury [12]. More broadly, other antibodies generated against lipids and possibly other antigens may benefit from FSA-CIR over traditional methods like ELISA for the evaluation of molecular interactions under near-physiological conditions.

Glossary

FSA: Free solution assay

CIR: Compensated Interferometric Reader

ELISA: Enzyme-linked immunosorbent Assay

LPA_1: Lysophosphatidic Acid Receptor 1

LPA: Lysophosphatidic Acid

LPC: lysophosphatidylcholine

PA: Phosphatidic Acid

RI: Refractive Index

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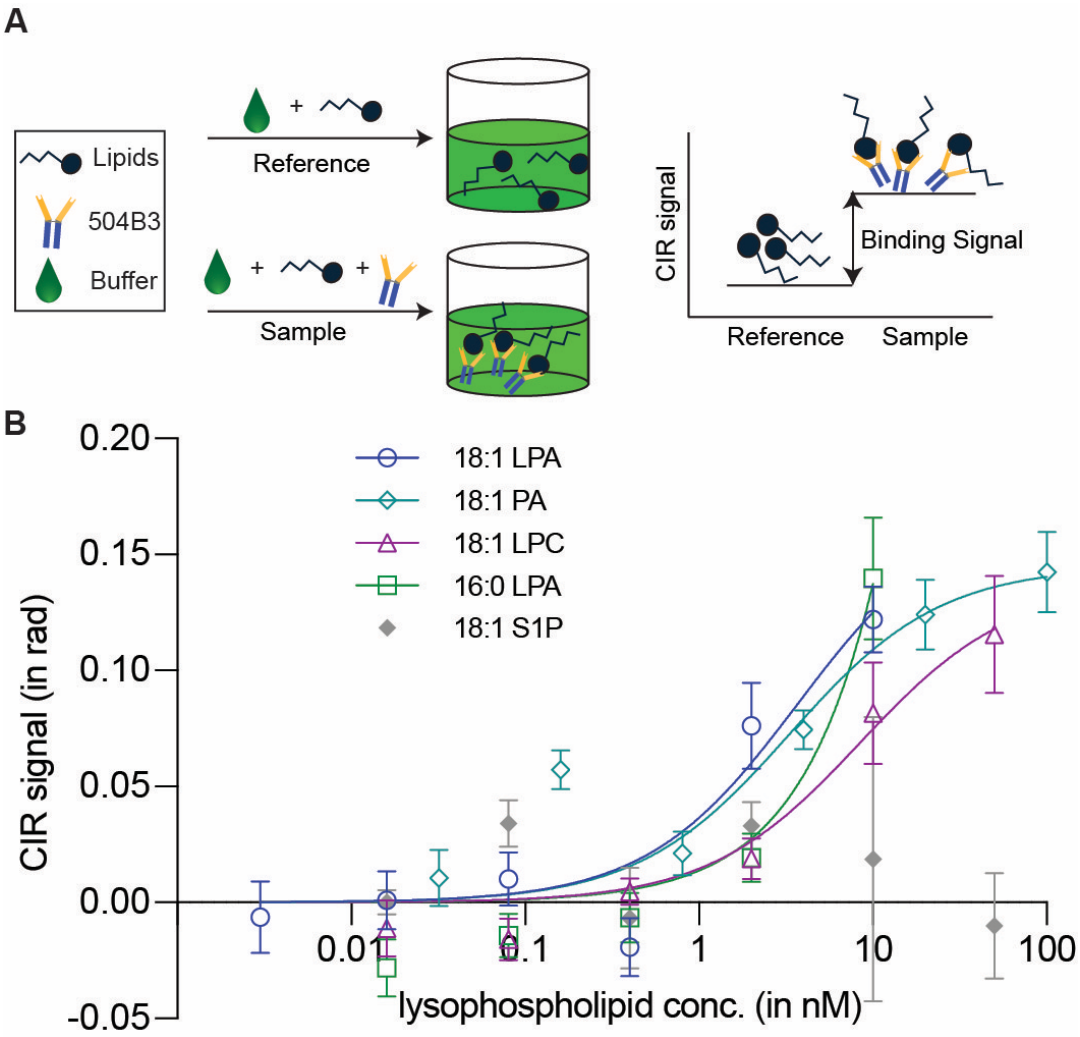
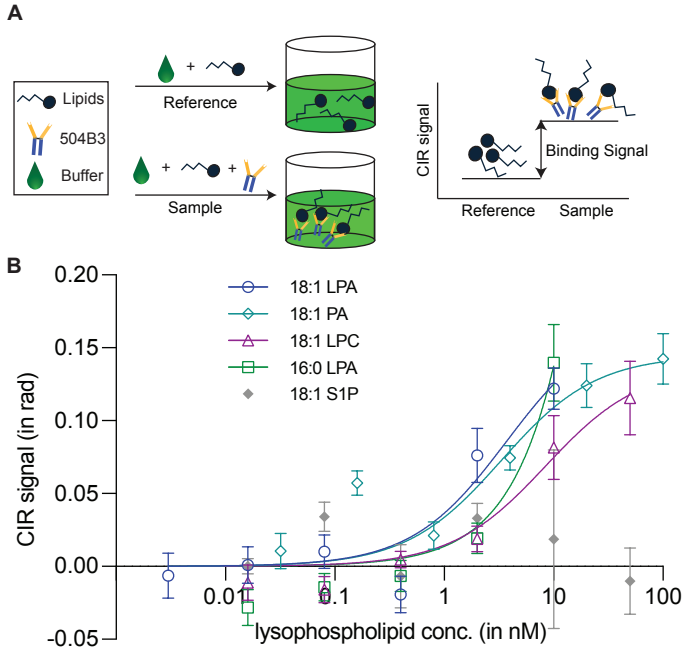


Figure 1: Free solution assay (FSA) measured by a compensated interferometric reader (CIR) to determine the binding constant (K_D) for 504B3 anti-LPA antibody against five lysophospholipid forms. (a) FSA used to prepare RI matched sample and reference solutions. *See supplementary information for detailed preparation methods.* (b) Binding curves for each LP or PA ligand (*e.g.,* 18:1 LPA, 18:1 PA, 18:1 LPC, 16:0 LPA and 18:1 S1P) used to determine the binding constants (K_D). Each graph shows an average of two independent binding isotherms (experimental replicates), each with quintuplicate measurements (technical replicates).


A
Lipids
504B3
Buffer
Reference
Sample
CIR signal
Binding Signal
Reference
Sample
B
18:1 LPA
18:1 PA
18:1 LPC
16:0 LPA
18:1 S1P
CIR signal (in rad)
0.20
0.15
0.10
0.05
0.00
-0.05
0.01
0.1
1
10
100
lysophospholipid conc. (in nM)