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Biophysical methods for the characterization of PTEN/lipid bilayer interactions

Rakesh K. Harishchandra [a], Brittany M. Neumann [a], Arne Gericke [a], Alonzo H. Ross [b,*]

[a] Worcester Polytechnic Institute, Department of Chemistry and Biochemistry, Worcester, MA 01605, USA
[b] University of Massachusetts Medical School, Department of Biochemistry and Molecular Pharmacology, Worcester, MA 01605, USA

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ABSTRACT

PTEN, a tumor suppressor protein that dephosphorylates phosphoinositides at the 3-position of the inositol ring, is a cytosolic protein that needs to associate with the plasma membrane or other subcellular membranes to exert its lipid phosphatase function. Upon membrane association PTEN interacts with at least three different lipid entities: An anionic lipid that is present in sufficiently high concentration to create a negative potential that allows PTEN to interact electrostatically with the membrane, phosphatidylinositol-4,5-bisphosphate, which interacts with PTEN's N-terminal end and the substrate, usually phosphatidylinositol-3,4,5-trisphosphate. Many parameters influence PTEN's interaction with the lipid bilayer, for example, the lateral organization of the lipids or the presence of other chemical species like cholesterol or other lipids. To investigate systematically the different steps of PTEN's complex binding mechanism and to explore its dynamic behavior in the membrane bound state, *in vitro* methods need to be employed that allow for a systematic variation of the experimental conditions. In this review we survey a variety of methods that can be used to assess PTEN lipid binding affinity, the dynamics of its membrane association as well as its dynamic behavior in the membrane bound state.

1. Introduction

The subcellular distribution of PTEN creates a fundamental problem to understanding its regulation and action. The most important substrate for PTEN is diacylphosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5)P_3), which is localized in the inner leaflet of the plasma membrane [1]. In contrast, PTEN is primarily localized in the cytoplasm where it freely diffuses [2]. Hence, in order to hydrolyze PI(3,4,5)P_3, PTEN must bind to the membrane and be activated. The first step is dephosphorylation of the PTEN tail (particularly sites S385, S380, T383 and T382). Sellers and coworkers proposed this influential model [3,4], positing that the phosphorylated tail folds back onto the PTEN core. This closed conformation blocks binding of PTEN to the membrane [5]. Dephosphorylation of the PTEN tail allows the second step, binding of PTEN to phospholipid membranes. Bound PTEN shows a shallow penetration of the membrane [6]; binding is dominated by interactions with the headgroups of negatively charged lipids [7,8]. An N-terminus domain preferentially binds diacylphosphatidylinositol-4,5-bisphosphate PI(4,5)P_2 [9,10]. The C2 domain binds negatively charged lipids, including phosphatidylserine (PS) [8]. The third step is a PTEN conformational change induced by PI(4,5)P_2. This conformation change activates the phosphatase domain [11] and was verified by infrared spectroscopy [9]. The fourth step is to diffuse laterally until it binds PI(3,4,5)P_3. The fifth step is hydrolysis of the phosphate in the 3-position of PI(3,4,5)P_3. Careful analysis of membrane-bound PTEN reveals that the lateral diffusion allows hydrolysis of multiple PI(3,4,5)P_3 molecules prior to the sixth step, release of PTEN from the membrane [12].

The goal of this review is to discuss biophysical methods for analyzing the kinetic and thermodynamic parameters for each step of PTEN activation. It should be emphasized that PTEN function is highly quantitative. Tumor formation can be enhanced by PTEN haploinsufficiency [13]. Hence, a quantitative understanding of

Abbreviations: ATR, attenuated total reflection; CD, circular dichroism; PI(3,4,5)P_3, diacylphosphatidylinositol-3,4,5-trisphosphate; PI(4,5)P_2, diacylphosphatidylinositol-4,5-bisphosphate; DOPIP, di-oleoyl; di-C_{16}, di-palmitoyl; FRET, fluorescence resonance energy transfer; FTIR, Fourier transform infrared; GUV, giant unilamellar vesicle; IR, infrared; LUV, large unilamellar vesicle; MWCO, molecular weight cutoff; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PIP, phosphoinositide; SUV, small unilamellar vesicle; SAPIPs, stearoyl-arachidonoyl; SPR, surface plasmon resonance.

* Corresponding author at: University of Massachusetts Medical School, Department of Biochemistry and Molecular Pharmacology, 364 Plantation Street Room 819, Worcester, MA 01605, USA. Fax: +1 508 856 2003.
E-mail address: Alonzo.Ross@umassmed.edu (A.H. Ross).

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PTEN regulation and membrane binding is essential to understand wild type and mutated PTEN proteins [14].

2. Biomembrane mimics for the characterization of membrane-associated PTEN

PTEN membrane association involves binding to multiple lipid entities. The C2 domain and probably other positively charged parts of the protein interact electrostatically with anionic lipids that generate a negatively charged environment at the water/membrane interface. Typically this role has been assigned to phosphatidylserine (PS) [9,15]. PTEN's N-terminal end interacts selectively with $PI(4,5)P_2$, which leads to enhanced binding and allosteric activation of the phosphatase [9]. Finally, the substrate $PI(3,4,5)P_3$ binds to the phosphatase domain active site. While $PI(3,4,5)P_3$ is in all likelihood the most important substrate *in vivo*, PTEN shows also *in vitro* activity towards the other 3-phosphorylated lipids. Interactions of other anionic lipids with the active site may contribute to PTEN membrane binding in the absence of 3-phosphorylated phosphoinositides. Considering PTEN's multifaceted lipid-binding properties, special consideration has to be given to the choice of the appropriate membrane model system and its composition. For a recent review on model membrane systems for the characterization of peripheral membrane proteins, please see Czogalla et al. [16].

2.1. Choosing the lipid composition

Phosphoinositides (PIPs) occur in nature almost exclusively with a stearoyl-arachidonoyl (SAPIPs) acyl chain composition. Since the acyl chain composition has a major impact on lipid phase behavior and hence lipid mobility as well as headgroup spacing, studies aimed at investigating phosphoinositide/PTEN interaction should utilize lipids with these acyl chains whenever possible. Phosphoinositides are also commercially available with di-oleoyl (DOPIPs) or di-palmitoyl acyl (di-C_{16} PIPs) chains. DOPIPs will form fluid phases, however, the phase behavior in the presence of other membrane components like cholesterol deviates slightly from what is observed for SAPIPs [17]. DPPIPs form ordered (gel) phases at room temperature [18], resulting in a reduced mobility and lateral spacing of di-C_{16}PIPs in comparison to SAPIPs and even though PTEN penetrates the bilayer only to a limited extent [19,20], bilayer model systems involving saturated chain phosphoinositides are less suitable to study PTEN membrane association. Short-chain PIPs (di-butanoyl or di-octanoyl PIPs are commercially available from a variety of vendors, see Table 1) are widely used to determine the activity of PTEN against soluble substrates. In some instances this test is used as a "quality control" measure to ensure that an expressed protein is active, while in some other cases the comparison of PTEN activity against soluble or vesicle resident $PI(3,4,5)P_3$ substrate highlights differences in interfacial vs. lipid bilayer independent activation. The critical micelle concentrations (CMCs) for short chain PIPs is around 0.5–1 mM [21] and typically experiments involving short chain PIPs are carried out at a concentration well below the CMC. However, others [21] have found some evidence that PTEN may promote PIP clustering through its various anionic lipid interaction sites, resulting in a significant lowering of the CMC like it was observed for other protein/amphiphile complexes. When interpreting data involving data obtained with soluble PIP lipids, this potential caveat should be kept in mind.

Phosphoinositides alone do not form stable vesicles and therefore, need to be mixed with other membrane constituents. To study the interaction of PTEN or PTEN derivatives with phosphoinositides (e.g., $PI(4,5)P_2$ or $PI(3,4,5)P_3$), phosphatidylcholine (PC) can be used as a "matrix" lipid to form vesicles since PTEN has a very small binding affinity for PC [9]. Alternatively, we have found that cholesterol stabilizes phosphoinositide vesicles if at least 10 mol% cholesterol is present [17]. While PTEN's interaction with $PI(4,5)P_2$ presumably involves hydrogen bonds between N-terminal amino acids and the inositol ring phosphomonoester groups, other parts of the protein have been found to interact non-specifically, electrostatically with anionic lipids [9]. To investigate synergistic lipid binding of PTEN, $PI(4,5)P_2$ can be mixed with PS or phosphatidylinositol, where the latter two will bind electrostatically, non-specifically to PTEN. The presence of cholesterol in $PI(4,5)P_2$ containing vesicles enhances PTEN binding, presumably due to cholesterol-induced $PI(4,5)P_2$ clustering [17].

The choice of the lipid composition is generally driven by the experimental goals. For example, experiments aimed at comparing the binding properties of wt and mutant PTEN may employ mixtures like brain $PI(4,5)P_2$/POPS/POPC (1:20:79). $PI(3,4,5)P_3$ hydrolysis can be measured by using binary PC/$PI(3,4,5)P_3$ with different lipid ratios (see below). To mimic plasma membrane inner leaflet lipid compositions, one might use lipid mixtures such as PE (33%)/PC (10%)/PS (21%)/PI (4.5%)/SM (4.5%)/cholesterol (25%)/PIPs (2%) [22], which is a frequently used model system. $PI(4,5)P_2$ concentration in the plasma membrane is about 1 mol%, while the other PIPs are found at lower concentrations [23].

2.2. Fluorescently and isotopically labeled phosphoinositides

Several types of experiments require the use of fluorescently labeled phospho inositides, which are available with different excitation/emission wavelengths from either Echelon or Avanti Polar Lipids (see Table 2). All of these phosphoinositide derivatives are labeled at the acyl chain, i.e., the PIP headgroup is non-derivatized. It should be noted that the fluorophore group for all of these PIPs is quite bulky, which might affect their organization in lipid bilayers. In general, chain labeled phospholipids will prefer fluid, disordered phases over liquid-ordered or gel phases. Furthermore, weak mutual phosphoinositide interaction, e.g., via hydrogen bond formation, cannot be effectively monitored with these labeled PIPs [17,24]. However, fluorescently labeled PIPs can be used very

Table 2
Commercially available fluorescently labeled phosphoinositides.

Fluorophore	Type	Excitation/emission	Vendor
BODIPY FL	PI & all PIPs	505/513 nm	Echelon
BODIPY TMR	PI & all PIPs	542/574 nm	Echelon
TopFluor	PI(4)P, $PI(3,5)P_2$ $PI(4,5)P_2$	495/503 nm	Avanti polar lipids

Table 1
Acyl chain composition of commercially available $PI(4,5)P_2$ and $PI(3,4,5)P_3$ lipids.

Lipid	di-C4	di-C8	di-C16	di-C18:1	C18:0/C20:4	Natural
$PI(4,5)P_2$	Various*	Various*	Various*	Avanti	Avanti	Avanti (brain)
$PI(3,4,5)P_3$	Various*	Various*	Various*	Avanti	Avanti	–

* Several vendors offer phosphoinositides with saturated acyl chains, including Ag Scientific, Alexis, Avanti Polar Lipids, Echelon, Matreya, Mobitech, Sichem, Sigma Aldrich.

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