

Bacterial Protein Phosphoinositide Probes

Specific Marker for PI(4)P

Invention

Phosphoinositide (PI) lipids are central second messengers in many cellular processes, including membrane trafficking, actin remodeling and receptor-mediated signal transduction. To date, specific probes to detect and quantify individual PI phosphates (PIPs) in biochemical or cell biological experiments are not available for all PIPs. Thus, in the scientific community, there is a high demand for probes specific for a given PIP.

Researchers at ETH Zurich discovered that the SidC and SdcA proteins secreted by the pathogenic bacterium *Legionella pneumophila* bind PIPs. *In vitro* GST-SidC (and -SdcA) fusion proteins directly and specifically bind to PI(4)P immobilized on nitrocellulose membranes, but not to other lipids and PIPs (Fig. 1A). A GST fusion protein of another secreted *L. pneumophila* protein (GST-SidD) did not bind any PIPs or other lipids under the same conditions. In infected host cells, SidC binds to the membrane of the *Legionella*-containing vacuole, which was determined using an affinity purified anti SidC antibody. Moreover, in the context of liposomes, GST-SidC also specifically binds PI(4)P but not PI(3)P or PI(4,5)P₂ (Fig. 1B). GST-SidD was used as a negative control in this assay. The PI(4)P-binding domain of SidC is located on a 30 kDa C-terminal fragment (data not shown) and is currently mapped at higher resolution. These features allow the use of tagged SidC (and SdcA) or fragments thereof as specific probes for PI(4)P in biochemical and cell biological assays.

Additional Information:

[Figure 1. Binding of GST-SidC to PI\(4\)P *in vitro*](#)

Keywords

Cell biology
Lipid-protein overlay assay
Phosphatidylinositol-4 phosphate
Phosphoinositide metabolism
Signal transduction
Vesicle trafficking

Patent Status

US Provisional Application

Competitive Advantages

- SidC specifically binds to PI(4)P but not to other PIPs or lipids.
- GST-SidC can be readily overexpressed in *E. coli* (e.g. strain BL21(DE3)) in a predominantly soluble form and purified by glutathione affinity chromatography. The yield is up to 25 mg/l LB medium and might be further optimized.
- Robust: GST-SidC is stable and withstands repeated cycles of freezing/thawing without loss of PI(4)P-binding activity.
- Moreover, the PI(4)P-binding domain is significantly smaller than, e.g., a PI(4)P-binding antibody.

Applications:

GST-SidC can be used as a PI(4)P probe in biochemical and cell biological assays:

- Detection and quantification of PI(4)P probe *in vitro*
- Use as a positive control in lipid-protein overlay or pull down experiments
- Staining of intracellular compartments in live or fixed eukaryotic cells
- Enrichment of PI(4)P-containing cellular compartments by pull down assays
- Ectopic expression of SidC, e.g. as a GFP fusion protein

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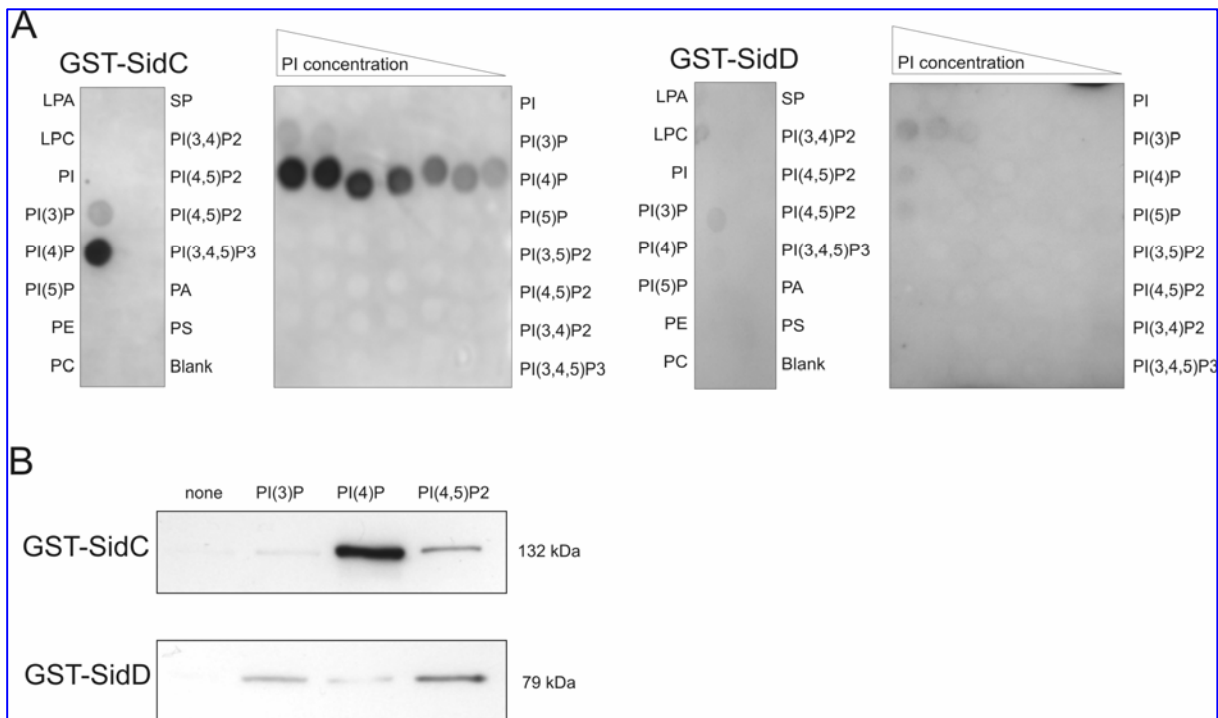


Figure 1. Binding of GST-SidC to phosphatidylinositol phosphates *in vitro*.

(A) A typical result from a lipid-protein overlay assay is shown. Binding of affinity-purified GST fusion proteins of SidC or SidD (160 pmol) to different lipids (100 pmol; left panels) or twofold serial dilutions of phosphoinositides (100 - 1.56 pmol; right panels) immobilized on nitrocellulose membranes was visualized using an anti GST antibody and chemiluminescence. The abbreviations are: phosphoinositides (PI), lysophosphatidic acid (LPA); lysophosphocholine (LPC), phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingosine-1-phosphate (SP), phosphatidic acid (PA), phosphatidylserine (PS).

(B) Phospholipid (PL) vesicles (20 μ l, 1 mM lipid) composed of PC (65%), PE (30%), and 5% (1 nmol) either PI(4)P, PI(3)P or PI(4,5)P₂, were incubated with affinity-purified GST-SidC or GST-SidD (40 pmol), centrifuged and washed. Binding of GST fusion proteins to PL vesicles was assayed by Western blot with an anti GST antibody.