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Phosphatidylinositol-Specific Phospholipase C (PI-PLC)

Introduction

Phosphatidylinositol-specific phospholipase C (PI-PLC) from *Bacillus cereus* (EC 3.1.4.10) cleaves the phospholipid phosphatidylinositol (PI) into two molecules: water-soluble *myo*-inositol 1:2-cyclic phosphate and lipid-soluble diacylglycerol (DAG).[1-3] This enzyme is a member of a large class of ubiquitous PI-PLCs. The smaller PI-PLCs (~35,000 MW), including *B. cereus* PI-PLC and the nearly identical *B. thuringiensis* PI-PLC, are secreted by bacteria.[4] The larger PI-PLCs exist in eukaryotes and generate second messengers in the PI signal transduction pathway.[4,5] *B. cereus* PI-PLC cleaves PI, lyso-PI and glycosylphosphatidylinositol (GPI)-containing structures in a calcium-independent manner.[4] Molecular Probes now offers a highly purified *B. cereus* PI-PLC (P-6466) suitable for both enzymology and cell biology applications.

Materials

B. cereus PI-PLC (P-6466) is supplied in a 50 µL volume at a concentration of 100 enzyme units/mL in 20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.01% sodium azide and 50% glycerol. When the enzyme is stored at -20°C, it should retain nearly full activity for at least six months. Over longer periods of storage at -20°C, the enzyme exhibits only minimal loss of activity; e.g., we have observed <30% loss of activity in 1.5 years.

Specifications

Molecular Probes' PI-PLC is a recombinant enzyme isolated from *Escherichia coli* transformed with the *B. cereus* PI-PLC gene.[6] The sequence of the gene is available as GenBank accession numbers M28549 and M30809.[7] The enzyme is purified by anion-exchange chromatography and is greater than 90% pure by SDS-polyacrylamide gel electrophoresis. The enzyme activity is determined using phosphatidylinositol (PI) as the substrate presented to the enzyme as mixed micelles with sodium deoxycholate.[1] For the assay, 20 µL of 10 mM [^{3}H]-PI is combined with 40 µL of 0.1 M HEPES-NaOH, pH 7.65 (25°C), 20 µL of 0.8% sodium deoxycholate and 20 µL of PI-PLC diluted to about 1 nM (35 ng/mL) in 0.1% BSA. The reaction is incubated for 10 minutes at 37°C; at this temperature the reaction will be at pH 7.5. The water-soluble, radiolabeled inositol phosphate reaction product is separated from unreacted PI substrate by extraction with 0.5 mL chloroform:methanol:concentrated HCl (66:33:1) and mixed with scintillation fluid for counting. One unit is the amount of enzyme that converts 1 µmol of substrate to product per minute under the conditions of the assay. Molecular Probes' enzyme preparations of this protein exhibit a specific activity greater than 1000 units per mg of purified protein; the specific activity (units per mg protein) of the individual lot is indicated on each vial.

Applications

B. cereus PI-PLC will cleave phosphatidylinositol in intact membranes and can be used to reduce the PI content of the outer membrane leaflet of intact cells. This activity has been employed in studies of PI synthesis and export to and across the plasma membrane[8] and to generate diacylglycerol, which itself activates protein kinase C and also serves as a substrate for other signaling enzymes.[9] Thus, bacterial PI-PLC is a useful tool in cell biological studies of the PI-dependent signal transduction pathway.

The *B. cereus* PI-PLC also provides an efficient means of releasing most GPI-anchored proteins from cell surfaces.[10-12] GPI-linked proteins are released under conditions in which the cells remain viable, allowing both isolation of the GPI-linked proteins and studies of the kinetics of their reappearance at the cell surface. One assay for GPI-cleaving activity involves the release of acetylcholinesterase from the plasma membrane of fresh bovine erythrocytes.[3] For release of GPI-linked proteins from cultured cells, we suggest the following protocol for a 60 mm diameter culture dish (containing approximately 0.5–1 × 10^6 cells). Rinse the cell culture twice with cold phosphate-buffered saline (PBS), then apply 0.5 mL of the same buffer containing 0.1–1.0 units of *B. cereus* PI-PLC and rock the culture at 4°C for 20 minutes. Recover the buffer for analysis of released proteins. Rinse the cell culture twice with PBS, add fresh medium and return to the incubator. The minimal amount of effective PI-PLC should be determined by an initial titration experiment with the actual target cell-type to be studied.

References

1. Methods Enzymol 197, 493 (1991); **2.** Biochemistry 29, 8056 (1990); **3.** FEMS Microbiol Lett 52, 237 (1989); **4.** J Mol Biol 275, 635 (1998); **5.** Science 244, 546 (1989); **6.** Protein Expr Purif 2, 51 (1991); **7.** J Bacteriol 171, 6077 (1989); **8.** Biochim Biophys Acta 1224, 247 (1994); **9.** J Biol Chem 269, 4098 (1994); **10.** J Neurochem 57, 67 (1991); **11.** J Biol Chem 266, 1926 (1991); **12.** Renal Physiol Biochem 16, 299 (1993).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
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