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ABSTRACT

Phospholipases are a class of enzymes that catalyze the hydrolysis of mem-
brane phospholipids. Phospholipase
Cys (PLCs) cleave phosphatidylinositol
phosphates (PIPs) into a diacylglycerol
lipid tail and an inositol phosphate polar
head group. These hydrolysis products
are crucial secondary messengers for
intracellular signaling and have been
implicated in several disease states.
PLCs are soluble proteins that anchor
to the cellular membrane by a phos-
phoinositide specific Pleckstrin Homol-
ogy (PH) domain. Once anchored to
the membrane, the catalytic X and Y
domains catalyze many cleavage re-
actions before dissociation. PLCs can
be classified as xi, beta, gamma, and
delta. PLCdelta binds PI(4,5)P2 with high
affinity. The crystal structure of the PH
domain revealed a positively charged
surface patch with high affinity inter-
tactions to the PI(4,5)P2 phosphates.

The high specificity of the PH domain
of PLCdelta was explored in several
experiments. Binding specificity was
shown qualitatively in a protein lipid
overlay with eight naturally occurring
phospholipids. PLCdeltaPH binding was
compared to a known promiscuous lipid
recognizing protein, LL5alpha. PLCdel-
taPH PI(4,5)P2 binding was optimized
quantitatively by chemi-luminescence
using an Alphascreen assay. Optimal
enzyme and substrate concentrations
were used in a competition Alphan-
screen assay. Five concentrations of
eight phosphoinositides were assayed
with constant PLCdeltaPH and PI(4,5)P2
conditions. PLCdeltaPH PI(4,5)P2 bind-
ing affinity in these varied lipid environ-
mets was quantified to see if the spe-
cific binding could be competed away.

The overlay showed a beautiful ar-
ray with PLCdeltaPH binding almost
exclusively to PI(4,5)P2 and LL5alpha
binding to nonspecifically many PIPs.
The Alphascreen showed maximum
binding occurring at 0.21 picomoles
PLCdeltaPH and 2.5 picomoles PI(4,5)P2
per well in a 2.5uL Alphascreen assay.

The competition assay demonstrated
the specific binding of PLC PH to PI(4,5)P2
can be competed away by other vari-
sous other PIPs. PI(3,4,5)P3, PI(4)P, and
PI(3,4)P2 the most effective inhibitors re-
spectively. PI(5)P and PI displayed minor
inhibition. PI(3)P and PI(3,5)P2 showed
no competition. It is reasonable to con-
clude that PIPs with a 4-phosphate were
better competitors than PIPs with a
5-phosphate. This suggests that the
interaction of the 4-phosphate is more
important than the 5-phosphate for the
specific binding of PLCdeltaPH to PI(4,5)P2.