Evidence for and Characterization of $Ca^{2\square}$ Binding to the Catalytic Region of *Arabidopsis thaliana* Phospholiparnss7T1

technique. PLD□ and PLD□cat demonstrated a marked ability to bind $Ca^{2\square}$ in the presence of phosphatidylserine (Fig. 1*C*) but not in its absence (data not shown). To obtain quantitative

In contrast, PIP_2 binding by PLD□ increased with $Ca^{2□}$ levels up to 100 □M concentrations of the cation (Fig. 6*B*). Further increases in $Ca^{2□}$ concentration sharply diminished the amount of PIP_2

has a similar but greater effect on PIP_2 binding to the whole enzyme (Fig. 6*B*), with the maximal value being attained at about 100 □M $Ca^{2□}$, a pattern resembling the $Ca^{2□}$-dependence of PLD□ activity (Fig. 2). Millimolar level $Ca^{2□}$

(38), temperature stress (39), and in response to a plant hormone (40). Both $Ca^{2\square}$ and PIP_2 function as cellular messengers in various cellular processes, and characterization of their direct interaction with PLD thus provides insights into the *in vivo* activation and function of $Ca^{2\square}$-dependent PLDs.

REFERENCES

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