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Activity of phosphatidylinositol-specific phospholipase C from *Bacillus cereus* with thiophosphate analogs of dimyristoylphosphatidylinositol

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Abstract

Phosphatidylinositol-specific phospholipase C (PI-PLC) was studied with sonicated dispersions of a thiophosphate analog of phosphatidylinositol, 1,2-dimyristoyloxypropane-3-thiophospho(1D-1-*myo*-inositol) (D-thio-DMPI). Kinetic parameters were derived from the rate as a function of bulk lipid concentration at constant saturating surface concentration of substrate (case I), and as a function of surface concentration of substrate at a constant saturating bulk concentration of lipid (case II). The substrate, D-thio-DMPI, was diluted with L-thio-DMPI or dimyristoyl phosphatidylmethanol (DMPM). In the presence of L-thio-DMPI, values for $V_{max} = 133$ μmol min^{-1} mg^{-1}, K'_s (the apparent dissociation constant for the enzyme-interface complex) = 0.097 mM, and K^*_m (the apparent interfacial Michaelis constant) = 0.22 mol fraction were obtained. DMPM caused enzyme inhibition in case I but no inhibition in case II. L-Thio-DMPI is an ideal neutral diluent with which to study the kinetics of PI-PLC. © 1997 Elsevier Science Ireland Ltd.

Keywords: *Bacillus cereus*; 1,2-Dimyristoyloxypropane-3-thiophospho(1D-1-*myo*-inositol); Phosphatidylinositol-specific phospholipase C; Thiophosphate; Interfacial kinetics

Abbreviations: D-Thio-DMPI, 1,2-dimyristoyloxypropane-3-thiophospho(1D-1-*myo*-inositol); L-Thio-DMPI, 1,2-dimyristoyloxypropane-3-thiophospho(1L-1-*myo*-inositol); DMPM, 1,2-Dimyristoyl-*sn*-glycerol-3-phosphomethanol; D-Thio-DPPI, 1,2-dipalmitoyloxypropane-3-thiophospho(1D-1-*myo*-inositol); DTP, 4,4′-dithiobispyridine; DTPM, 1,2-ditetradecyl-*sn*-glycerol-3-phosphomethanol; HDPC, hexadecylphosphocholine; MES, 2-(*N*-morpholino)ethanesulfonic acid; PC, phosphatidylcholine; PI, phosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C.

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1. Introduction

Phosphatidylinositol-specific phospholipase C (PI-PLC; EC 3.1.4.10) from *Bacillus cereus* catalyzes the cleavage of phosphatidylinositol to diacylglycerol and 1D-*myo*-inositol-1,2-(cyclic)phosphate. The cyclic phosphate is subsequently slowly hydrolyzed by the same enzyme to 1D-*myo*-inositol-1-phosphate (Volwerk et al., 1990; Griffith et al., 1991; Zhou et al., 1997). This enzyme also catalyzes the release of a number of enzymes and other proteins linked to glycosylphosphatidylinositol membrane anchors (Low and Saltiel, 1988). PI-PLC in mammalian cells plays a key role in signal transduction through the hydrolysis of phosphatidylinositol bisphosphate to two second messengers, inositol trisphosphate and diacylglycerol (Rhee et al., 1989; Dennis et al., 1991). For a recent review of PI-PLC see Bruzik and Tsai (1994).

Early studies on the enzymatic properties of the bacterial enzyme were reported by Sundler et al. (1978). They found no requirement for divalent cations and demonstrated inhibition by high concentrations of NaCl. More detailed mechanistic studies have shown that the formation of the cyclic phosphate occurs with inversion of the phosphorus configuration, and the formation of the final product, *myo*-inositol-1-phosphate, with overall retention of configuration (Lin et al., 1990; Bruzik et al., 1992). The X-ray crystal structure of PI-PLC from *B. cereus* suggests that the mechanism involves general acid and base catalysis similar to that of ribonuclease (Heinz et al., 1995). The substrate must have a free 2-hydroxyl group since the 2-*O*-methyl analog of phosphatidylinositol is not hydrolyzed (Lewis et al., 1993), and only the isomer with 1D-*myo*-inositol is hydrolyzed; isomers with 1L-*myo*-inositol are neither substrates nor inhibitors (Volwerk et al., 1990; Lewis et al., 1993; Leigh et al., 1992).

PI-PLC, like other water-soluble phospholipases, is subject to 'interfacial activation', the preference for aggregated over monomeric substrate. This was shown by Hendrickson et al. (1992) for various PI analogs, and by Lewis et al. (1993) for short-chain PI. This activation, in which the activity increases several fold, may involve a change in enzyme conformation (Zhou et al., 1997; Volwerk et al., 1994). The simplest mechanism involves reversible binding of the soluble enzyme to the lipid interface, followed by an interfacial Michaelis–Menten mechanism (Fig. 1).

Kinetic studies of PI-PLC have been hampered by the lack of a continuous simple assay with phospholipids closely resembling the natural substrate in bilayer membranes. A water-soluble substrate analog, *myo*-inositol-1-(4-nitrophenylphosphate) (Shashidhar et al., 1991) was used in a kinetic analysis of PI-PLC (Volwerk et al., 1994). Since this substrate does not form micelles nor readily incorporate into micelles or bilayers, its use is mostly limited to a study of the enzyme in the absence of an interface. We have developed thiophosphate analogs, with sulfur in the bridging position between the glycerol carbon and phosphorus. This facilitates a continuous assay when coupled to the colorimetric detection of free thiol (Fig. 2). The first of these analogs, a simple *n*-alkyl inositol thiophosphate, was used in monomolecular and micellar systems (Hendrickson et al., 1991, 1992). However, the activity with PI-PLC was only about 10% that with the natural substrate, and bilayer structures could not be formed. We then reported the synthesis of an optically-active thiophosphate analog of DMPI, D-thio-DMPI (see Fig. 2), that may form bilayer structures (Hendrickson et al., 1996). It has an activity with PI-PLC about one-third that with the natural substrate. Recently Hondal et al. (1997) reported the use of a dipalmitoyl thiophos-

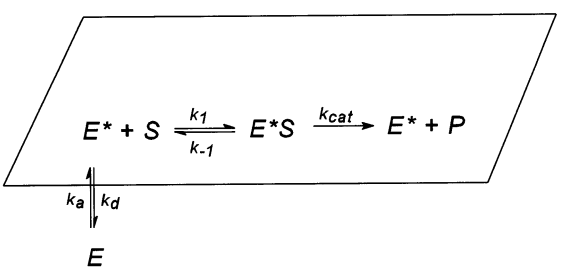

Fig. 1. Scheme to illustrate a reaction mechanism for PI-PLC. The species (E*, enzyme; S, substrate; and P, products) shown in the box are within the two-dimensional lipid interface. The soluble enzyme in the aqueous phase is shown as E.

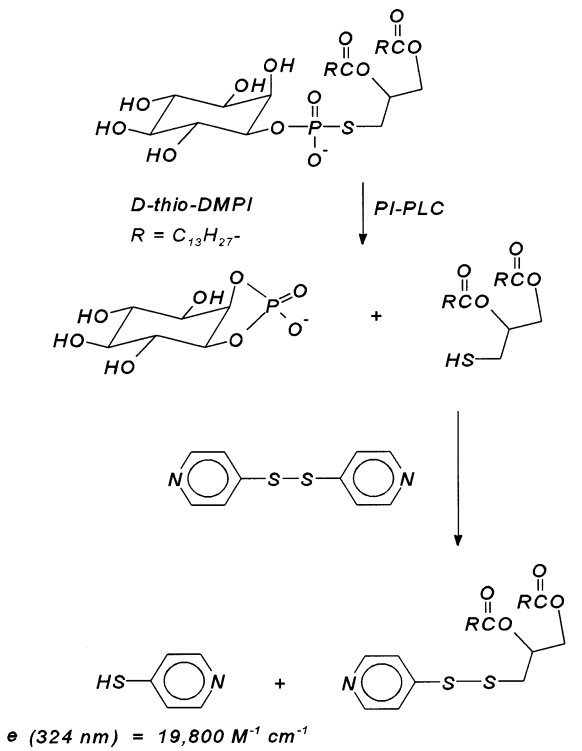

Fig. 2. Reaction scheme for the assay of PI-PLC using D-thio-DMPI as the substrate.

phate analog of PI, D-thio-DPPI, for the assay of PI-PLC. It did not readily disperse in water and required detergents for its use. In mixed micelles with diheptanoyl PC, it gave only about half of the activity of sonicated dispersions of D-thio-DMPI with PI-PLC. Kinetic studies of PI-PLC with D-thio-DMPI, the non-substrate stereoisomer L-thio-DMPI, and DMPM are reported here.

2. Materials and methods

2.1. Materials

D-Thio-DMPI and L-thio-DMPI were synthesized as described by Hendrickson et al. (1996). HDPC was synthesized as described by van Dam-Mieras et al. (1975). DMPM and DTPM were generous gifts from Mahendra Jain, University of Delaware. MES, Triton X-100, and *S*-carboxymethyl bovine serum albumin were obtained from Sigma, St. Louis, MO. DTP (Aldrithiol-4) was obtained from Aldrich, Milwaukee, WI.

Recombinant *B. cereus* PI-PLC was expressed in *Escherichia coli* and purified by the procedure of Koke et al. (1991) using the pIC expression plasmid (a generous gift from J.J. Volwerk, University of Oregon). The protein concentration was determined using an extinction coefficient of 1.84 ml mg^{-1} cm^{-1} at 280 nm (Volwerk et al., 1994). Dilute solutions of enzyme were stabilized in 0.1% *S*-carboxymethyl bovine serum albumin.

2.2. Lipid dispersions

Stock solutions of phospholipids in chloroform–methanol were stored at −20°C. The concentration of phospholipid was determined by phosphorus analysis (Eaton and Dennis, 1976). Aliquots were dried under a stream of nitrogen and then under high vacuum. MES buffer (50 mM, pH 7) was added and the dispersions were vortexed and sonicated briefly in a bath-type sonicator (Lab Supplies, Hicksville, NY). The dispersions were then frozen and sonicated until thawed; this was repeated at least once, after which an optically-clear dispersion was obtained.

2.3. Enzyme activity

PI-PLC activity was determined by the spectrophotometric method described by Hendrickson et al. (1996) (see Fig. 2). Briefly, the lipid was sonicated and freeze-thawed in 50 mM MES buffer, pH 7; this procedure was repeated once again. DTP (1 mM) and enzyme were added (final volume, 235 μl) and the absorbance at 324 nm was recorded at 30°C. The time courses were initially linear with little or no lag. Activity was calculated using an extinction coefficient for thiopyridine of 19 800 M^{-1} cm^{-1} (Yu and Dennis, 1991). Kinetic data were fitted to Hill modifications of the Michaelis–Menten equation (see Section 3.2, Eqs. (1) and (2)) by non-linear least-squares regression analysis using the program Sigma Plot (Jandel, San Rafael, CA).

3. Results

3.1. Complete hydrolysis of lipid dispersions of d-thio-DMPI

Thio-DMPI was readily dispersed in buffer by sonication and freeze-thawing. These dispersions were optically clear. A sonicated dispersion of D-thio-DMPI was completely hydrolyzed with PI-PLC in the presence of DTP. The time course for the reaction is shown in Fig. 3. The data fitted poorly (dotted line) to a single first-order rate equation, but much better (solid line) to the double first-order rate equation, $A = A_1\ (1 - e^{-k_1 t}) + A_2(1 - e^{-k_2 t})$, where $A_{max} = A_1 + A_2$. The initial absorbance increase up to 200 s fitted well (dashed line) to a single first-order rate equation with a maximal absorbance of 0.697. By contrast, mixed-lipid micelles of D-thio-DMPI and HDPC were completely hydrolyzed at a faster rate with a time course (Fig. 4) that fit well to a single first-order rate equation (expected at low substrate concentration in the first-order region of the Michaelis–Menten rate curve). In both cases the maximum observed experimental absorbance was 1.12. Theoretical absorbance for complete hydrolysis of substrate is 1.14; this indicates an optical purity of 98% D-thio-DMPI. Complete hydrolysis of L-thio-DMPI under similar conditions showed that it contained about 8% D-thio-DMPI.

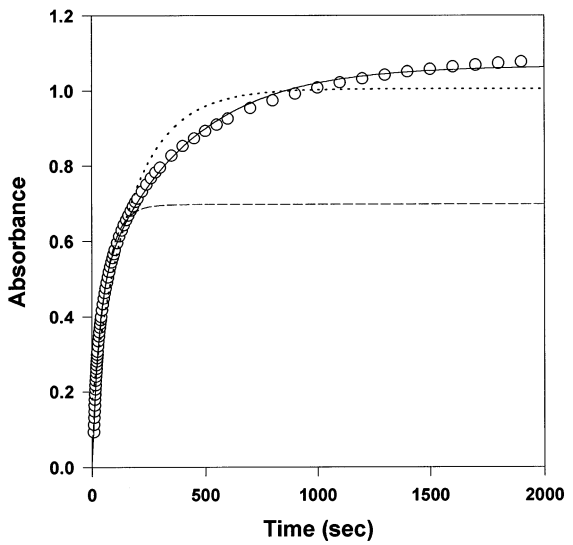

Fig. 3. Time course for PI-PLC-catalyzed hydrolysis of a sonicated dispersion of D-thio-DMPI. D-Thio-DMPI, 57.4 μM; PI-PLC, 0.48 μg; total volume, 235 μl; $T = 30$°C. Open circles: experimental data; solid line: theoretical curve for data fitted to a double first-order rate equation ($k_1 = 0.0334\ s^{-1}$, $k_2 = 0.00261\ s^{-1}$, $A_{max} = 1.07$); dotted line: theoretical curve for data fitted to a single first-order rate equation ($k_1 = 0.00581\ s^{-1}$, $A_{max} = 1.00$); dashed line: theoretical curve for data up to 200 s fitted to a single first-order rate equation ($k_1 = 0.0184\ s^{-1}$, $A_{max} = 0.697$).

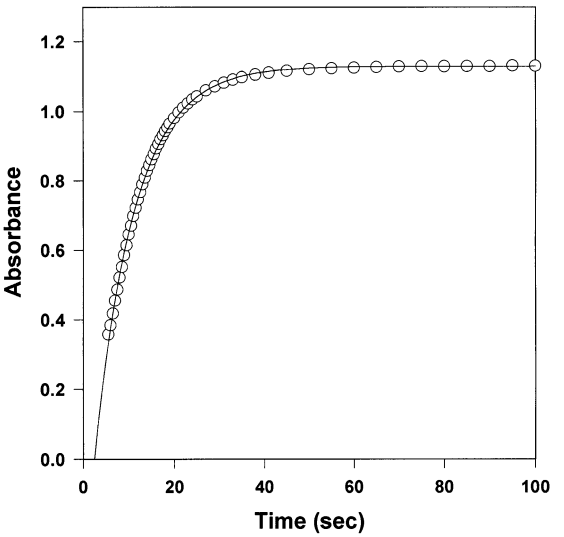

Fig. 4. Time course for PI-PLC-catalyzed hydrolysis of mixed-lipid micelles of D-thio-DMPI and HDPC. D-Thio-DMPI, 57.4 μM; mol ratio HDPC/D-thio-DMPI, 4.0; PI-PLC, 0.48 μg; total volume 235 μl; $T = 30$°C. The enzyme was added a few seconds after zero time. Open circles: experimental data; solid line: theoretical curve for data fitted to a single first-order rate equation ($k_1 = 0.114\ s^{-1}$, $A_{max} = 1.12$).

3.2. Kinetic analysis

The simplest model for interfacial catalysis by PI-PLC involves reversible binding of the soluble enzyme to the lipid interface, characterized by a dissociation constant $K_s = k_d/k_a$, followed by an interfacial Michaelis–Menten mechanism in a two-dimensional system, characterized by an interfacial Michaelis constant, K^*_m, and a maximal velocity V_{max}. This is illustrated in Fig. 1.

Kinetics can be studied in mixed-lipid aggregates by two types of experiments (Hendrickson

and Dennis, 1984). In case I, the surface concentration of substrate, X_s, is held constant by a fixed ratio of substrate to non-substrate lipid, and the rate of reaction measured as a function of the bulk concentration of total lipid. This follows a Michaelis–Menten-type equation (Hendrickson and Dennis, 1984, Eq. 7) where the concentration of lipid at half-maximal velocity is equal to an apparent dissociation constant $K'_s = K_s/(1 + X_s/K^*_m)$. The actual dissociation constant, K_s, is a function of the apparent dissociation constant, the surface concentration of substrate, and K^*_m, since binding of substrate to the enzyme active site will shift the preceding equilibrium to the right. The apparent $V'_{max} = V_{max}/(1 + K^*_m/X_s)$. If the mol fraction of substrate, X_s, is much greater than the apparent interfacial Michaelis constant (the interfacial enzyme is saturated with substrate), the experimental maximal velocity is equal to V_{max}.

In case II, the bulk concentration of total lipid is held constant while the surface concentration of substrate is varied by changing the ratio of substrate to non-substrate lipid. The rate also follows a Michaelis–Menten equation with respect to surface concentration of substrate. The apparent $K^{*\prime}_m = K^*_m(1 + K_s/[\text{lipid}])$. If the bulk concentration of total lipid is much greater than the value of K_s (the enzyme is saturably bound to the interface), the experimental maximal velocity is equal to V_{max}, and the surface concentration at half-maximal velocity is equal to the apparent K^*_m.

Non-hyperbolic, sigmoidal kinetic curves are often seen when there is cooperativity in binding or when allosteric effects occur. This is empirically formulated in a modified form of the Michaelis–Menten equation known as the Hill equation, where the concentration and Michaelis constant terms are raised to the h power. A Hill coefficient, h, greater than one reflects positive cooperativity. The following equations were used to fit kinetic data in the two types of experiments:

$$v = \frac{V_{max}[\text{lipid}]^h}{K_s^h + [\text{lipid}]^h} \quad (1)$$

Case I at constant saturating surface concentration of substrate.

$$v = \frac{V_{max}X_s^h}{K_m{*}^h + X_s^h} \quad (2)$$

Case II at constant saturating bulk concentration of lipid.

3.3. Kinetics of PI-PLC with sonicated phospholipid dispersions

Enzyme activity as a function of bulk concentration of total lipid at a constant surface concentration of substrate (case I) is shown in Fig. 5. The kinetic constants are given in Table 1. Both racemic and optically-active (not shown) thio-DMPI gave pronounced sigmoidal curves with Hill coefficients between 3 and 5. The apparent V_{max} was somewhat greater (although not twice that of racemic thio-DMPI) for D-thio-DMPI, suggesting that this may be approaching a saturating value. DMPM at 0.5 mol fraction had a great effect on increasing the value of K'_s, while only slightly lowering the apparent V_{max}. DMPM, thus, appears to lower the affinity of the enzyme for the interface while not significantly changing the interfacial catalytic activity. It also showed high apparent cooperativity.

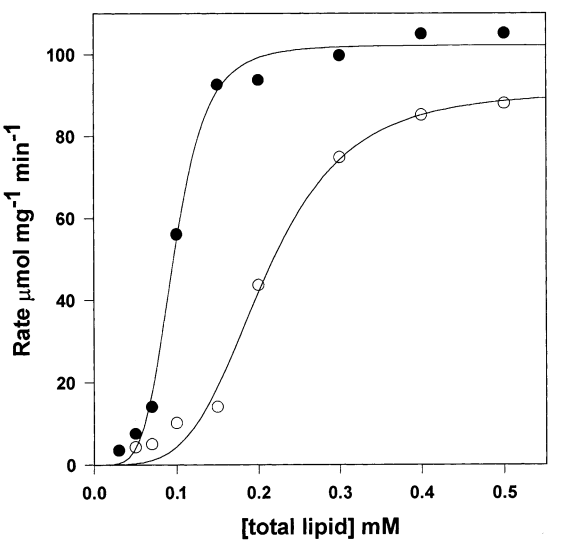

Fig. 5. Activity of PI-PLC as a function of bulk concentration of lipid (case I). Closed circles, racemic thio-DMPI; open circles, 0.5 mol fraction of D-thio-DMPI in DMPM; 11.8 ng (1.46 nM) PI-PLC; 50 mM MES, pH 7; $T = 30$°C. Data fitted to Eq. (1).

Table 1
Kinetic constants for PI-PLC-catalyzed hydrolysis of D-thio-DMPI

Non-substrate lipid	Constant surface or bulk concentration	V_{max} (μmol min^{-1} mg^{-1})	Ks' (mM)	K^*_m (mol fraction)	h
Case I					
None	$X_s = 1.0$ mol fraction	133 ± 2	0.093 ± 0.002		3.0
L-thio-DMPI	$X_s = 0.5$ mol fraction	102 ± 2	0.097 ± 0.003		4.8
DMPM	$X_s = 0.5$ mol fraction	91.1 ± 5.0	0.208 ± 0.011		4.1
Case II					
L-thio-DMPI	[Lipid] = 0.9 mM	130 ± 12		0.22 ± 0.04	1.0
DMPM	[Lipid] = 0.9 mM	129 ± 13		0.17 ± 0.05	0.8

Kinetic data were fitted to Eq. (1) or Eq. (2) by non-linear regression. V_{max}, apparent maximal velocity; K'_s, apparent dissociation constant for enzyme–interface complex; K^*_m, apparent interfacial Michaelis–Menten constant; h, Hill coefficient; X_s, surface concentration of substrate (D-thio-DMPI).
The errors are shown as S.D., defined as the square root of the mean of the square of the differences from their mean of the data samples for each curve in Figs. 5 and 6.

Enzyme activity as a function of surface concentration of substrate at a constant saturating bulk concentration of lipid (case II) is shown in Fig. 6. The kinetic constants are given in Table 1. D-Thio-DMPI diluted with L-thio-DMPI gave a hyperbolic curve with an apparent V_{max} similar to that seen in case I with D-thio-DMPI. The apparent interfacial Michaelis constant was 0.22 mol fraction. D-Thio-DMPI diluted with DMPM gave very similar results indicating that DMPM does not inhibit the catalytic activity of PI-PLC when all of the enzyme is bound to the interface. Similar results (not shown) were also seen with DTPM.

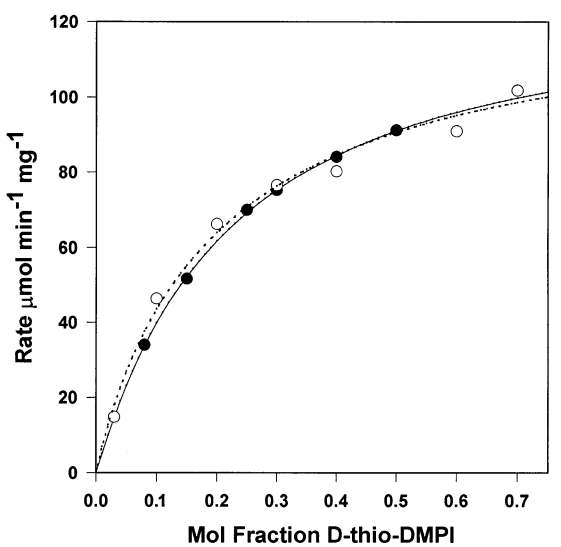

Fig. 6. Activity of PI-PLC as a function of surface concentration of D-thio-DMPI. Closed circles–solid line, D-thio-DMPI in L-thio-DMPI; open circles–dotted line, D-thio-DMPI in DMPM; 23.7 ng (2.92 nM) PI-PLC; bulk lipid concentration, 0.9 mM; 50 mM MES, pH 7; $T = 30$°C. Data fitted to Eq. (2).

4. Discussion

Thio-DMPI, with two 14-carbon fatty acid chains per head group, should form lipid bilayer structures. This lipid was readily dispersed in buffer by sonication to give an optically-clear dispersion. Phospholipid–detergent mixed micelles at low substrate concentration were 98% hydrolyzed in a single first-order rate process (Fig. 4). Pseudo first-order kinetics would be expected at low substrate concentrations in the first-order region of the Michaelis–Menten rate curve. Dispersions of D-thio-DMPI without detergent were 98% hydrolyzed by PI-PLC, but the kinetics seemed to be at least biphasic (Fig. 3). That would be expected for a closed bilayer structure that slowly opens up to expose more substrate. It could also be due to a polydisperse substrate (see discussion below). These assays showed that D-thio-DMPI was 98% pure, and L-thio-DMPI was 92% pure (contains 8% D-isomer).

Lewis et al. (1993) observed that the time course for hydrolysis of short-chain PI micelles by PI-PLC leveled off after only 20–30% hydrolysis. This, they suggested, might be due to product inhibition. James et al. (1996) observed similar time-dependent inhibition of PI hydrolysis by mammalian phospholipase Cβ in phospholipid vesicles and phospholipid-detergent mixed micelles, but not in phospholipid monolayers at an air-water interface. They ascribed this inhibition to product-dependent 'alteration of the character' of the lipid interface rather than enzyme inactivation or product inhibition. We see none of these effects; if there is product inhibition, it is only weak. However, the product of D-thio-DMPI hydrolysis in the presence of DTP is a more phospholipid-like molecule than diacylglycerol, with a dithiopyridinium head group, and may have less perturbing effects on the character of the interface. Also, product dissociation from the enzyme should be much different.

The kinetics of D-thio-DMPI hydrolysis as a function of total lipid concentration (case I) showed pronounced apparent cooperativity (sigmoidal curves), with Hill coefficients between 3 and 5 (Fig. 5). The cause of this cooperativity is not clear. Zhou et al. (1997) also see apparent cooperativity in the kinetics of PI-PLC cleavage of PI and the hydrolysis of *myo*-inositol-1,2-(cyclic)phosphate. While there is no evidence for aggregation of the protein, and the crystallized protein is clearly monomeric (Heinz et al., 1995), there may be lipid-induced protein aggregation or binding of lipid to an allosteric site resulting in a conformational change to a more-active form of the enzyme. Zhou et al. (1997) showed 'interfacial activation' of the enzyme towards hydrolysis of the water-soluble cyclic phosphate by the presence of lipid micelles or vesicles. Volwerk et al. (1994) also observed activation of the enzyme-catalyzed hydrolysis of the water-soluble substrate, *myo*-inositol-1-(4-nitrophenylphosphate), in the presence of a lipid interface. These results argue strongly for a conformational change in the enzyme, since only the enzyme, but not the water-soluble substrate, interacts with the interface.

There are other possible explanations for the apparent cooperativity we observe. (a) It may be due to phase separation or some type of reorganization of lipid within the interface with increased lipid concentration. If this occurs, and the enzyme favors a phase enriched in substrate or a more favorable interfacial organization of lipid, increasing amounts of that phase with increasing lipid concentration would show increased activity. (b) It may be due to polydispersity of substrate particle size. If the substrate consists of two or more different particle sizes, where each particle exhibits the same V_{max} but different K_s values, then the rate equation becomes a polynomial function of bulk lipid concentration and could appear as a sigmoidal curve. We plan to characterize the lipid dispersions in order to better understand this apparent cooperativity.

DMPM greatly reduces the apparent affinity for enzyme binding at the interface, but has little effect on the maximal velocity. This appears to be the main inhibitory effect of DMPM, since we see little or no effect of DMPM on interfacial kinetics in case II (see below). Again, this effect may simply be due to phase separation or reorganization of lipids within the interface in the presence of DMPM. The kinetics of D-thio-DMPI hydrolysis as a function of surface concentration of substrate at constant saturating bulk lipid concentration (case II) showed normal Michaelis–Menten behavior, with no cooperativity, in the presence of L-thio-DMPI or DMPM (Fig. 6). DMPM caused no inhibition in this case, when all of the enzyme was bound to the interface.

Volwerk et al. (1994) showed evidence for competitive inhibition of PI-PLC by DTPM. Our failure to see any competitive inhibition by either DMPM (Fig. 6) or DTPM after enzyme binding to the interface, argues against simple competitive inhibition at the active site. The inhibition seen by Volwerk et al. (1994) (Fig. 10) may simply be due to decreased binding of enzyme to the interface caused by the addition of DTPM, if the bulk concentration of lipid was not sufficient to saturate the interface with enzyme. They used a bulk concentration of PI (2 mM) which may not be sufficiently greater than the apparent bulk K_m. Their calculated value for K_i^* of 0.045 mol fraction for DTPM, compared to their calculated value for K_m^* of 0.26 mol fraction for PI, seems

unreasonable in view of the many hydrogen-bonding contacts seen in the crystal structure of the PI-PLC–*myo*-inositol complex (Heinz et al., 1995). Substituting a methyl group for the inositol moiety would be expected to result in much weaker binding at the active site. In any case, it appears from our work that DMPM acts by inhibiting enzyme binding to the interface, but it has no effect on the interfacial mechanism after the enzyme is bound to the interface in case II. After many repeated experiments with both DMPM and DTPM, we simply see no evidence in case II for competitive inhibition at the active site by lipids with this head group.

L-DMPI seems to be an ideal 'neutral diluent' (lipids that form an interface to which the enzyme binds, but which do not bind at the active site (Jain et al., 1991)) for kinetic studies with PI-PLC; its physical properties are identical to those of D-thio-DMPI, but it is not able to bind at the enzyme active site. PC, as used by Volwerk et al. (1994) and Hondal et al. (1997), is less suitable as a neutral diluent. Since it has no net charge, varying its surface concentration with respect to PI will significantly change the membrane surface charge, which may affect interfacial binding of the enzyme (Mosior and McLaughlin, 1992) and also catalytic activity (Boguslavsky et al., 1994). In addition, we see significantly different kinetic behavior of PI-PLC with D-thio-DMPI in the presence of PC (Giles and Hendrickson, unpublished report), such as reduced apparent cooperativity in case I and significant apparent cooperativity in case II, which we cannot explain.

What seems clear is that PI-PLC follows a two-stage kinetic mechanism, where binding of the enzyme to the lipid interface precedes an interfacial Michaelis–Menten mechanism. Our results give an apparent interface dissociation constant (K'_s) and apparent interfacial Michaelis–Menten parameters (K^*_m and V_{max}) with thio-DMPI dispersions (Table 1). Enzyme binding to the interface (case I) shows apparent cooperativity, suggesting interfacial activation possibly due to a conformational change in the enzyme; alternatively, this may be due to lipid phase separation or reorganization within the interface, or polydispersity of lipid particle size. Recent studies by Zhou et al. (1997) strongly support a conformational change involved in the phosphodiesterase activity with the water-soluble cyclic-inositol phosphate substrate; this may also be true with the water-insoluble lipid substrates in the phosphotransferase reaction (Volwerk et al., 1994). Whether such a conformational change is the result of lipid-induced enzyme aggregation or the stabilization of an active form of the enzyme by substrate binding at an allosteric site(s) is still an open question.

More work needs to be done in order to understand the mechanism of PI-PLC action at a lipid interface. The nature of 'interfacial activation' is still not understood and needs further investigation, particularly with respect to the roles of non-substrate lipids and a possible conformational change in the enzyme upon binding to the interface. Also, the rate-limiting step in the catalysis needs to be defined. A study of the element effect (thio-DMPI versus DMPI) on the rate of the reaction may implicate the bond-breaking step as being rate limiting (Jain et al., 1992).

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