Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 2, pp. 49-72, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0968-0896/94 $6.00 + .00

0968-0896(93)E0010-L

REVIEW ARTICLE

Toward the Mechanism of Phosphoinositide-Specific Phospholipases C

Karol S. Bruzik*[a] and Ming-Daw Tsai*[b]

[a]*Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, U.S.A.*

[b]*Departments of Chemistry and Biochemistry, The Ohio State University, 120 West 18th Ave., Columbus, OH 43210, U.S.A.*

Introduction

Receptor-mediated turnover of inositol phospholipids and inositol phosphates has received unparalleled attention during the last decade (*ca* 2000 papers a year) owing to its importance in cellular signal transduction. The key enzyme responsible for triggering the cascades of such metabolic events is phosphatidylinositol-specific phospholipase C (PI-PLC, EC 3.1.4.10; CA Registry Number 63551-76-8).[1–14] This review is aimed at understanding molecular details of the structure, function, and catalytic mechanism of PI-PLC from mammalian and bacterial sources. Another very important aspect of PI-PLC—the mechanism of regulation—is not included since it has already been addressed in many recent reviews.[7,9,11–21]

The common feature of the various forms of PI-PLC is that they cleave the phosphodiester moiety of PI and its phosphorylated or glycosylated derivatives (**1a–d**) to produce the corresponding cyclic 1,2-inositol phosphates (**2a–d**) or the mixtures of cyclic (**2**) and acyclic 1-phosphates (**3a–d**) (Figure 1). Several products of the enzymatic cleavage of inositol phospholipids, including inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), have been proposed to act as calcium-mobilizing second messengers delivering the original extracellular message carried by a hormone, neurotransmitter or growth factor inside the cell.

Abbreviations: DAG, 1,2-diacyl-*sn*-glycerol; DPPI, 1,2-dipalmitoyl-*sn*-glycero-(1D-1-phospho-*myo*-inositol); DPPsI, 1,2-dipalmitoyl-*sn*-glycero-(1D-1-thiophospho-*myo*-inositol); GIP, glycosylated IP; GlcP, glycosylated lcP; GPI, glycosylphosphatidylinositol; Gro, glycero; IP, (I-1-P) 1D-*myo*-inositol 1-phosphate; IC_{50}, inhibitor concentration causing 50% inhibition of activity; IcP, 1D-*myo*-inositol 1,2-cyclic phosphate; IcPs, 1D-*myo*-inositol 1,2-cyclic phosphorothioate; IP_3, 1D-*myo*-inositol 1,4,5-trisphosphate; mfVSG, membrane form Variant Surface Glycoprotein; PA, phosphatidic acid; Pal, palmitoyl; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PI-4,5-P_2 (PIP_2), phosphatidylinositol-4,5-bisphosphate; PI-4-P (PIP), phosphatidylinositol-4-phosphate; PI-PLC, phosphatidylinositol-specific phospholipase C; SDC, sodium deoxycholate.

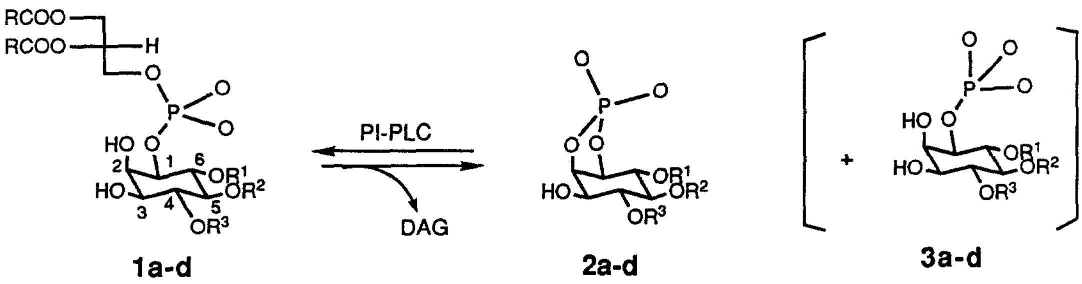
A subclass of PI-PLC, glycosylphosphatidylinositol-specific phospholipase C (GPI-PLC), catalyzes cleavage of the spacer arm of GPI-anchored proteins to release extracellular enzymatic activities of these proteins.[22–34] Structural variations of GPI (**4**, Figure 2), include the presence of glucosamine[95] or galactosamine[97,98] and the attachment of a varying number of phosphoethanolamine groups and oligosaccharide branches of the glycan main chain.[24,33,95,99] Although the majority of naturally occurring GPIs are derivatives of *myo*-inositol as shown in Figure 2, some *chiro*-GPIs have also been found in nature.[96–98] Analogous cleavage of the membrane constituent GPIs generates water-soluble phosphoinositol glycans implicated in the regulation of a variety of insulin-sensitive enzymes.[35–40] The substrate preferences and other characteristics of various enzyme types are summarized in Table 1.

PI-PLCs show no structural or functional similarity to the nonspecific PLC. The latter prefers phosphatidylcholine and also accepts a variety of phospholipids. PI-PLCs, on the other hand, do not hydrolyze phospholipids other than PI at any substantial rate. *B. cereus* PLC is a zinc metalloenzyme with three Zn^{2+} ions.[41] No sequence homology is found between three related enzymes from *B. cereus:* PI-PLC, nonspecific (or PC-specific) PLC, and sphingomyelin-specific PLC.[42]

Types of PI-PLC

(1) Mammalian PI-PLC

Mammalian PI-PLC[1–15,42–79] cleave PI (**1a**) and its phosphorylated forms PI-4-P (**1b**) and PI-4,5-P_2 (**1c**) to produce a mixture of inositol 1,2-cyclic (**2a–c**) and acyclic phosphates (**3a–c**).[64,65,79,80] The preference of PI-PLCs for phosphorylated or nonphosphorylated substrates depends on the protein type and the level of calcium (Table 1). Typically, PIP_2 is a preferred substrate at low (physiological) concentrations of calcium ion while PI becomes a preferred substrate at millimolar calcium concentrations. Several PI-PLCs such as the enzymes from guinea pig[58] and melanoma cell line[73] are active in the



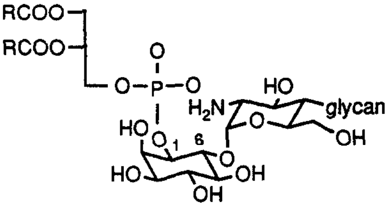
R = C_nH_{2n+1}, DAG = diacylglycerol

Figure 1. The reactions catalyzed by PI-PLC
1a–3a (PI, IcP, I-1-P): $R^1 = R^2 = R^3 = H$. **1b–3b** (PIP, IcP-4-P, I-1,4-P_2): $R^1 = R^2 = H$, $R^3 = PO_3^{2-}$. **1c–3c** (PIP_2, IcP-4,5-P_2, I-1,4,5-P_3): $R^1 = H$, $R^2 = R^3 = PO_3^{2-}$. **1d–3d** (GPI, GlcP, GIP): R^1 = glycosaminoglycan, $R^2 = R^3 = H$.

absence of calcium and are only mildly activated by calcium. These enzymes either prefer the nonphosphorylated PI (melanoma PI-PLC[73]) or hydrolyze various PI derivatives with similar efficiency (guinea pig PI-PLC[81]). The preference for a specific type of fatty acid is low.[82]

(2) Bacterial PI-PLC

Bacterial PI-PLCs[42,83–93] cleave nonphosphorylated (**1a**) and glycosylated forms (**1d**) of phosphatidylinositol and lysophosphatidylinositol.[30,85,87,88] They are much more efficient catalysts than mammalian enzymes (with V_{max} 1000–2000 versus 15–30 μmol-mg^{-1}-min^{-1};[57,88] see Table 1).



4

Figure 2. Structure of glucosamine glycan containing GPI (**4**).

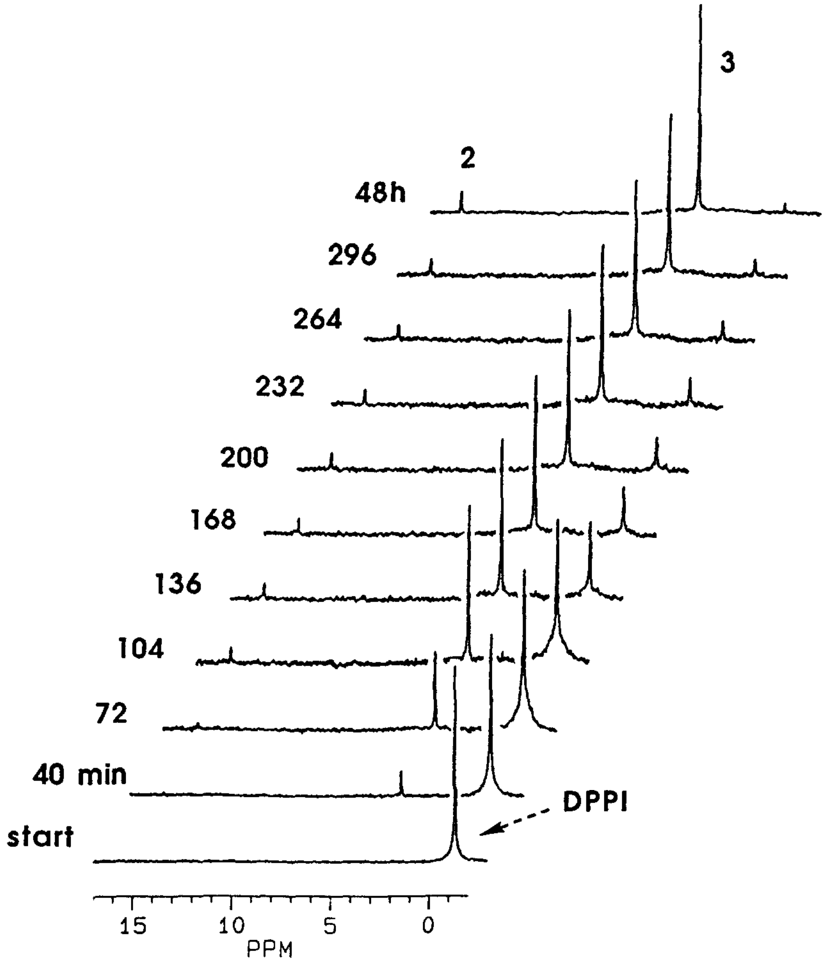


Figure 3. Time course of the hydrolysis of [^{16}O, ^{17}O]DPPI catalyzed by bovine brain PI-PLC-β_1 at pH 8, followed by ^{31}P NMR (from ref. 94 with permission).

Table 1. Properties of purified and partially purified PI- and GPI-PLC from various sources

Source	Enzyme type[a]	Size (kDa)[b]	Substrate preference	$K_{m,app}$ (mM)[c]	Specific Activity[c] (µmol·mg^{-1}·min^{-1})	Metal Ion (K_m or half-max. effect)	Reference
Mammalian PI-PLC							
bovine brain							
	α	60[d]	PIP_2/PIP	nd	8[i]	Ca^{2+}	61
	α	57	PIP_2/PI	0.09/0.26	0.005/0.25	Ca^{2+}	76
	$β_1$	154	PIP_2/PIP>PI	0.16/0.25[h]	>100	Ca^{2+} (5-10 µM)[i]	239
	$γ_1$	148					52, 239
		85	PIP_2>PI	nd	30-40	Ca^{2+} (10-100 µM)[i]	48
	$δ_1$	85	PIP_2>>PI[f,g]	0.096/0.144[h]	24	Ca^{2+} (0.6 µM)	46, 243
bovine heart							
	PIC3	nd	PIP_2/PIP/PI			Ca^{2+} (230 nM)	115
	δ	85	PIP_2/PI	0.095/0.0001[h]	6.1/0.076[h]	Ca^{2+} (9.1/1.7 nM[h])	149
bovine liver							
	β	150 (PI-PLC-M_1)[j]	PIP_2>>PI	0.015-0.05 (sat. [i])	nd	Ca^{2+} (0.025-0.1 µM)	75
bovine ROS							
		nd	PIP_2>>PI	nd	nd	Ca^{2+}(0.1-0.01 mM)	146, 250
		nd	PIP_2>>PI	nd	nd	Ca^{2+}(0.1-0.01 mM)	250
bovine smooth muscle cells							
	α	58	PIP_2/PI	nd	204/29	Ca^{2+}(0.17/0.3 mM)	151a
		157	PI>>PIP_2	nd	nd	Ca^{2+} (0.09 mM)	151a
bovine spleen							
	$γ_2$	145	PIP_2/PI	0.16/0.11	12.8/18.1	Ca^{2+} (0.1 mM)	67
guinea pig							
	α	58	PIP_2/PIP/PI	0.1/0.04/0.01	7.1/3.2/0.7	Ca^{2+}	58
human spleen							
		56	PI[k,l]	0.005	nd	none	74
		18	PI[k]	0.0015	nd	Ca^{2+} (0.5-1 mM)[i]	74

Table 1. *Continued*

human melanoma							
		150	PI>PIP_2	0.2/0.2	52/11	Ca^{2+} (3 mM)[i]	73
platelets							
	mPLC-II	63 (m)	PIP_2> PI	0.5/0.7	8.6/1.3	Ca^{2+} (5-10 μM)[i]	44
	mPLC-I	69 (m)	PIP_2> PI	0.5/0.7	9.3/1.3	Ca^{2+} (5-10 μM)[i]	44
	PLC-II	57 (c)	PIP_2> PI	0.4/0.07	7.8/2.6	Ca^{2+} (5-10 μM)[i]	44
		67 (c), 120 (c), 70 (c)	PIP_2, PI			Ca^{2+} (0.1-10 μM)	251
	mPLC-II	61 (m)	PIP_2> PI			Ca^{2+} (0.1-10 μM)	60
	γ_2	145 (c)	PIP_2, PI			Ca^{2+} (10μM, 1 mM)	252
	β	150 (m)	PIP_2>>PI	0.12/0.29	38.9/1.6	Ca^{2+} (10-100 μM)	150
	β, fragment	100 (c)	PIP_2>>PI	0.11/0.23	40.5/1.8	Ca^{2+} (10-100 μM)	150
		140[n]	PIP_2, PIP, PI	nd	nd	Ca^{2+} (10-100 μM)[o]	56
		98	PIP_2/PI	0.01-0.05[o]	1.2-2.5[o]	Ca^{2+} (Mg^{2+}, [o])	62
		440 (3x146)[d,e]	PIP_2			Ca^{2+}	69
porcine brain							
	β	145 (c)	PIP_2			Ca^{2+} (0.0001-0.01)	68
porcine lymphocyte							
		175[m]	PIP_2/PIP	nd	nd	Ca^{2+} (1 mM)[i]	49
rabbit brain							
	β_m	155 (c)	PIP_2> PI	nd	4[o]	Ca^{2+} (0.5 μM[o])	51
	IV	66, 61, 54[m]	PIP_2> PI	nd	0.3[o]	Ca^{2+} (0.5 μM[o])	51
rat brain							
	δ	85	PIP_2	0.06[p]	15.3[p]	Ca^{2+} (1-10 μM)	50
			PIP	0.075[p]	3.3[p]	Ca^{2+}	50
			PI	>0.2[p]	2.7[p]	Ca^{2+} (>1 mM)	50
	δ	85	PIP_2	0.13[p]	12.9[p]	Ca^{2+} (ca. 10 μM[o])	50
			PIP (not PI)	0.08[p]	6.5[p]	Ca^{2+}	50
		160	PIP_2, PIP, PI			Ca^{2+}	253
	ε	86	PIP_2 (not PI)				245
rat liver							
		87(c)	PIP_2=PIP>>PI	0.087[o]	2390[o]	Ca^{2+} (2 μM[o])	59
sheep seminal vesicles							
	α	65 (c)	PI>>PG	0.02-0.05	24.7, 28.7	Ca^{2+}	234
		85	PI>>PG	0.02-0.05	7.5	Ca^{2+}	234
turkey erythrocytes							
		150	PIP_2/PIP	nd	nd	Ca^{2+}	70

Table 1. *Continued*

wheat						
		PIP_2/PIP (not PI)			Ca^{2+} (Mg^{2+})	113
			Bacterial PI-PLC			
B. cereus						
	34.5	PI/GPI-AP[q]	1.3 (PI)		none	83, 42
		PI/GPI-AChE[r]	2[s]/0.017[r]	1660[s]/56[s]/0.3[r]	none	42, 102, 87, 116
B. thuringiensis						
	34.5	PI		1850	none	86, 254, 87, 255
C. novyi						
	30	PI/GPI-AP	nd	nd	none	84
Listeria monocytogenes						
	32.9	mfVSG/PI	0.15[i]	nd	none	90, 246, 256
Cytophaga sp.						
	17	PI, GPI-prot.	2		none	92
	22	PI, GPI-AChE	0.2	nd	none	147
S. aureus						
	33[t]	PI, GPI-prot	nd	nd	none	89,257,258
			GPI-PLC			
Trypanosoma brucei						
	39 (m)	mfVSG>> PI[a]	0.004[i]	2	none	105,110
	37 (m)	mfVSG, lipid A>> PI			none	103
	40 (m)	GPI, mfVSG	nd	nd	none	106
rat liver						
	52 (m)	GPI, mfVSG	nd	nd	none	107
rat serum	nd	GPI-AChE[r]	0.00016		Ca^{2+} (10 μM)[i]	102

[a]as defined in ref. 4; [b]c and m in parentheses stand for cytosolic and membrane-bound, respectively; [c]determined with substrates shown; [d]native protein is a trimer; [e]dimeric protein is also detected; [f]PI is utilized only at high [Ca^{2+}]; [g]PIP_2 hydrolysis is inhibited at high [Ca^{2+}]; [h]values for PIP_2 and PI, respectively; [i]saturating conc; [j]PI-PLC-M_1 and M_2 were shown to be of β type but more responsive to stimulation; [k]activity with PIP_2 was not determined; [l]complex with a regulatory protein; [m]several activities with similar molecular weight; [n]three other proteins with molecular weights of 95 and 270 and 400 kDa with similar substrate and calcium specificity were also isolated; [o]with PIP_2 as substrate; [p]at 0.1 mM Ca^{2+}; [q]GPI-anchored alkaline phosphatase; [r]GPI-anchored acetylcholinesterase as a substrate; [s]PI as a substrate; [t]molecular weight of 20 kDa was also reported

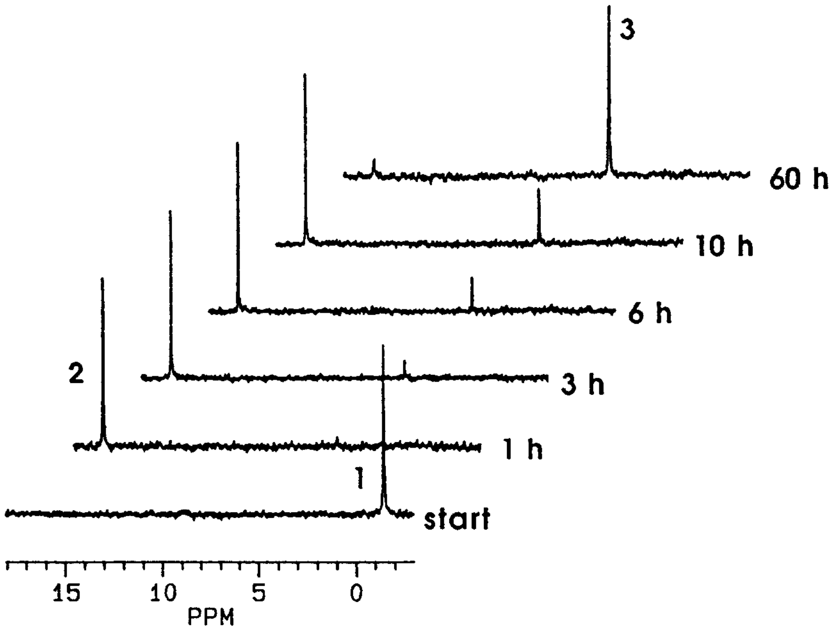


Figure 4. Time course of the reaction of DPPI catalyzed by *B. cereus* PI-PLC monitored by ^{31}P NMR. Sample conditions: DPPI (4 μmol) and PI-PLC (3.3 μg) in 0.1 M triethylammonium bicarbonate buffer, pH 7.0, containing 2% Triton X-100 and 5mM EDTA. Compounds **2** and **3** are IcP and IP, respectively. The numbers given to the right of each spectrum correspond to the midpoint of the accumulation period (from ref. 94 with permission).

While mammalian PI-PLCs produce both cyclic and acyclic inositol phosphates simultaneously (Figure 3), bacterial enzymes produce only IcP and diacylglycerol. However, PI-PLC from *B. cereus* and *B. thuringiensis* have been shown to further hydrolyze IcP at low rates to give inositol 1-phosphate (IP)[93,94] (Figure 4) which may explain an earlier finding of both cyclic and acyclic products.[83] Another difference between bacterial and mammalian PI-PLC is that phosphorylated PIs are not substrates for bacterial PI-PLC.[88,91] Furthermore, bacterial PI-PLC may be used as a tool to release GPI-anchored proteins from the membrane.[30] The primary products of the cleavage of GPI is presumed to be the corresponding 1,2-cyclic phosphate and diacylglycerol,[29,95] therefore palmitoylation at the 2-hydroxyl group of the inositol makes GPI-anchors resistant to PI-PLC cleavage.[99–101] With P1-PLC from *B. cereus,* binding of GPI anchors is *ca* 50–100 times stronger than that of PI while their turnover is 10^2 times slower thereby maintaining almost constant V_{max}/K_m.[102]

(3) GPI-specific PLC (GPI-PLC) from Trypanosoma brucei.[32,103–106]

This variant of PLC is involved in the removal of the variant surface glycoprotein during the life cycle of the parasite.[24,32] The preferred substrates for these enzymes are GPI (**1d**) and GPI–protein conjugates such as membrane form of Variant Surface Glycoprotein (mfVSG)[105] or their corresponding lyso-derivatives.[106]

(4) Insulin-stimulated mammalian GPI-PLC

This enzyme[107–110] is implicated in the mechanism of the transduction of the insulin signal.[23,39,40,107] GPI-PLC appears to have a very stringent structural requirement for glycosylated derivatives of phosphatidylinositol. Marginal cleavage of PI (**1a**) has been observed with the trypanosomal enzyme,[105,110] but not with rat liver enzyme.[107] Phosphorylated PIs (**1b,c**) are not substrates either.[107] The fact that GPI-PLCs utilize structurally heterogeneous mfVSG as a substrate[95,105] suggests that some modifications in the glycan chain are allowed.

In addition to these four types, PI-PLC has also been isolated from other sources such as *Dictyostelium*,[111] *Drosophila*[112] and plants.[113–115] The PI-PLC activity has also been detected and characterized in many animal tissues without isolation. The enzymes in the first two categories constitute the majority of PI-PLCs. Many of these proteins have been isolated, sequenced and characterized[9,30] and a few have been overexpressed.[104,105,116]

Chemical Mechanism of PL-PLC Reactions

(1) Parallel versus sequential formation of IcP and IP

The most intriguing mechanistic question for PI-PLCs is why mammalian PI-PLCs produce both cyclic and acyclic products simultaneously[117] (Figure 3) while in the case of bacterial PI-PLCs the reaction is clearly sequential (Figure 4).[93,94] Two other enzymes, annexin III, which utilizes glycero-1-phosphoinositol,[118,119] and the nonspecific phospholipase C from *Listeria*,[120] which utilizes phosphatidylinositol, produce exclusively acyclic inositol 1-phosphate as their final product. Whether the cyclic 1,2-phosphate is involved as an intermediate in the reactions of these two enzymes remains to be determined. For mammalian PI-PLC the ratios between cyclic and acyclic products depend on the substrate (**1a–c**), isozyme (β–δ), pH, and calcium concentration.[79] In the case of isozymes from bovine brain the percentage of the cyclic phosphates decreases in the orders PLC-β > PLC-δ > PLC-γ and PI > PIP > PIP_2.[79] The latter is also true for the α-isozyme from sheep seminal vesicles.[64] The proportion of cyclic phosphates increases at lower pH.[64,121] The increase of calcium concentration above the threshold value of 2 mM abruptly increases the percentage of cyclic phosphates when PIP and PIP_2 (but not PI) are the substrates.[79]

The most straightforward explanation for the simultaneous formation of the dual product (IcP + IP) by mammalian PI-PLC is a competitive attack of the water molecule or the inositol 2-hydroxyl group at the phosphorus atom[122] (Figure 5, Scheme A). This could be the consequence of several mechanistic possibilities: (i) there is an actual competition between water molecule and the hydroxyl group in the single active site, which would require an unlikely scenario with simultaneous activation of the water molecule and the hydroxyl group; (ii) there are two conformational states of the enzyme–substrate complex, one using water and the other using hydroxyl group as the attacking nucleophile; and (iii) there are two active sites on the same protein each carrying out a different reaction. An alternative explanation is that the two products are formed in two consecutive steps (Figure 5, Scheme B) as in the case of bacterial enzymes, but part of the IcP intermediate is released from mammalian enzymes. In Schemes A and B we have assumed direct nucleophilic displacements. In principle, the reactions could also go through an enzyme–IP intermediate **5** as shown in Schemes C and D in Figure 5.

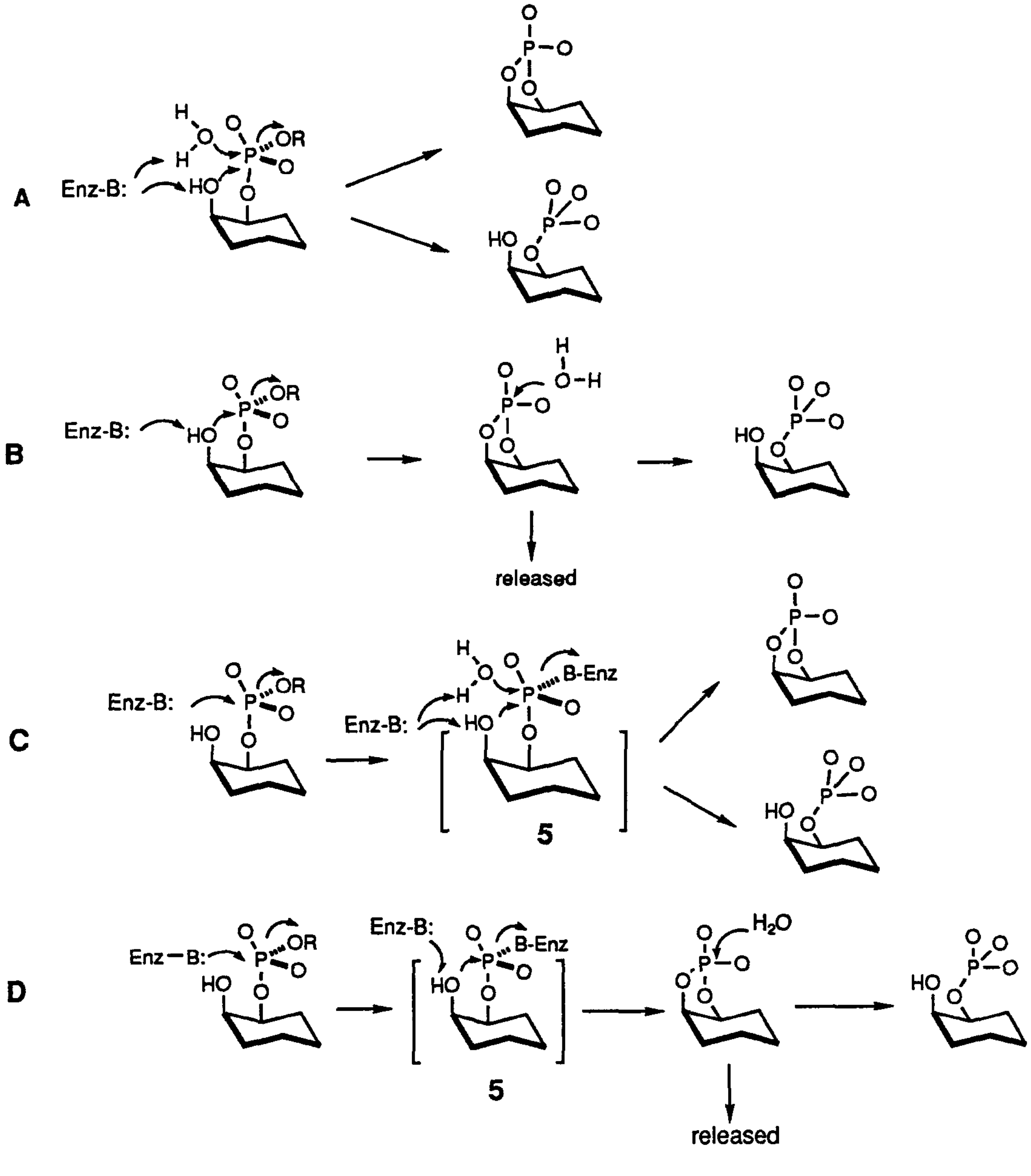


Figure 5. Possible mechanisms of PI-PLC.

Distinction between the four different mechanisms in Figure 5 was made by determining separately the steric courses of the formation of IcP and IP from the P-stereogenic analogs of phosphatidylinositol **6** and **7** in Figure 6.[94,123–126] The underlying stereochemical principle is that, barring pseudorotation, a single displacement should result in the inversion of configuration at the phosphorus atom while an even number of steps proceeding with inversion should lead to overall retention.[127,128] As shown in the Scheme A of Figure 7, cleavage of R_p-**6** (a preferred substrate over S_p-**6**) by both guinea pig PI-PLC-α and *B. cereus* PI-PLC occurs with inversion of configuration at phosphorus to give *trans*-IcPs (**8**).[123,124] In a separate series of experiments (Figure 7, Scheme B), cyclization of P-chiral diastereomers of [^{16}O, ^{17}O]DPPI (**7**) to [^{16}O,^{17}O]IcP (**9**) catalyzed by PI-PLC-β$_1$ was also shown to proceed with inversion of configuration.[94] These results rule out mechanisms C and D in Figure 5.

Hydrolysis of diastereomers of [^{16}O,^{17}O]IcP (**9**) to [^{16}O,^{17}O, ^{18}O]IP (**10**) by *B. cereus* PI-PLC proceeded with inversion of configuration as expected for a single displacement step.[94] Since IcP is an incompetent substrate for mammalian PI-PLC, the same reaction cannot be examined directly. However, formation of [^{16}O,^{17}O, ^{18}O]IP from [^{16}O,^{17}O]DPPI catalyzed by bovine PI-PLC-β$_1$ was shown to proceed with overall retention. This result strongly suggests that in spite of the deceptively simultaneous formation of IcP and IP by mammalian

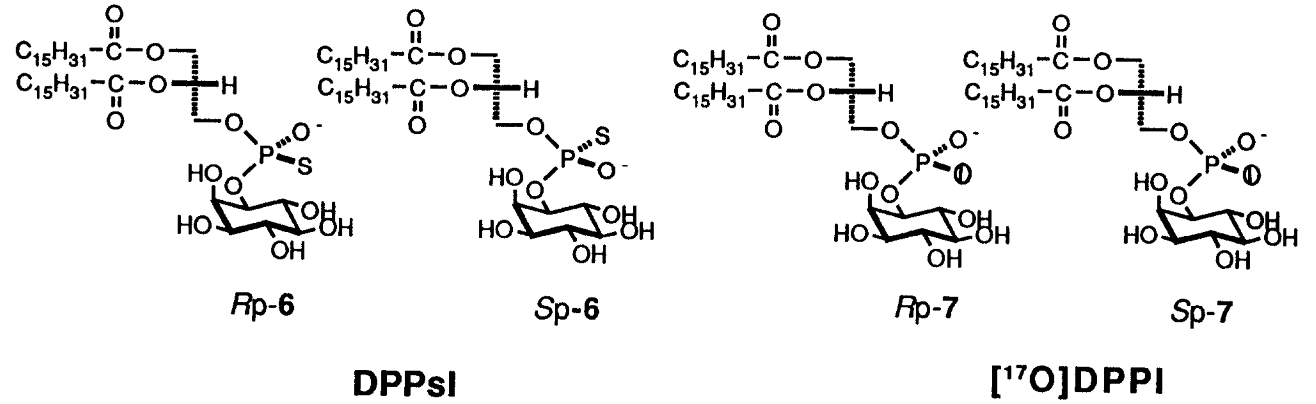


Figure 6. Structures of R_p and S_p isomers of DPPsI and [^{17}O]DPPI.

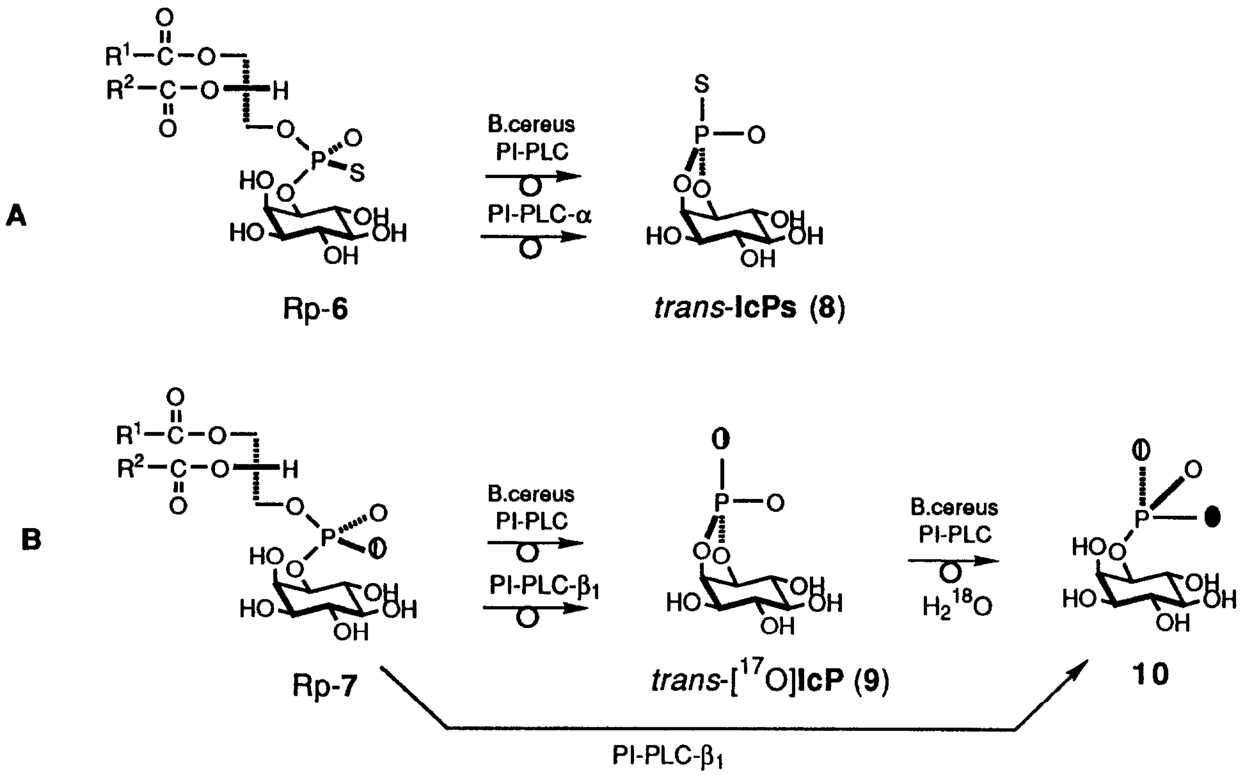


Figure 7. Stereochemical courses of the reactions catalyzed by PI-PLC.

enzymes this process is likely to be sequential (Scheme B in Figure 5), in agreement with the mechanism of bacterial enzymes. The only difference between mammalian and bacterial enzymes could be in the relative rates of the microscopic steps. The kinetic incompetence[129] of IcP for mammalian PI-PLC could be explained by its inability to partition into the micelles or bilayers of the substrate or to bind productively due to the lack of a hydrophobic tail. Even with bacterial enzymes IcP is hydrolyzed only at a very low rate.

(2) Transesterification of IcP with primary alcohols

The reversibility of enzymatic reactions is frequently utilized for synthetic purposes (e.g. base exchange activity of phospholipase D).[130,131] For bacterial PI-PLC it has been demonstrated that formation of IcP from PI is a reversible process in which primary alcohols can substitute for diacylglycerol to form the acyclic diester (Figure 8). Formation of acyclic diesters was observed with a number of primary alcohols such as methanol, propanol, Tris buffer, ethylene glycol, propane 1,2-diol, glycerol, D-mannitol, L-serine, and choline, but not with secondary alcohols, as components of the reaction mixture.[132] The new diester **11** is a much poorer substrate than PI and therefore accumulates in the course of the process. The product from glycerol consists of an equimolar mixture of *sn*-1 and *sn*-3 derivatives. The broad scope of acceptable primary alcohols and the low stereospecificity of transesterification are consistent with the lack of glycerol C-2 stereospecificity of the PI cleavage[94] and suggests that the alcohol binding site is relatively flexible.

Catalytic Properties and Cofactors

(1) Interfacial binding and detergents

PI-PLC are enzymes which utilize membrane-bound substrates in their natural environment. Most PI-PLC (with notable exception of the species from *S. aureus*) prefer micellar substrates dispersed with detergents such as sodium deoxycholate, Triton X-100 or octyl glucoside.[50,59,87,90,134,139,140,142] However, the majority of the kinetic results on PI-PLC obtained to date have not dealt with the problem of 'interfacial binding'.[133] Furthermore, the results obtained under different substrate conditions may be difficult to compare, particularly since some of the PI-PLCs are cytosolic proteins and some are membrane-bound. Several PI-PLCs show different pH maxima when acting on micellarly dispersed, vesicular and monolayer substrates or substrates dispersed with different detergents.[90,122,134] The cytosolic sheep seminal PI-PLC is activated by diglycerides and fatty acids, but inhibited by PC.[78,134,135] On the other hand, the human platelet enzyme (membrane-bound) is greatly activated by phosphatidic acid (PA), but not by diglycerides nor by other phospholipids.[136] The effect of PA on PI-PLC-γ_1 depends on whether the enzyme is in its activated (tyrosine phosphorylated) or latent form.[137] The effect of PA on the phosphorylated form is to decrease the K_m, while it is acting as an allosteric modifier on the unphosphorylated form. The minimal concentration of substrates required to observe hydrolysis with PI-PLC-δ_1 decreases in the order: di-C_4-PI > di-C_6-PI > di-C_8-PI, consistent with the decrease in their critical micelle concentrations.[138] PI-PLC-

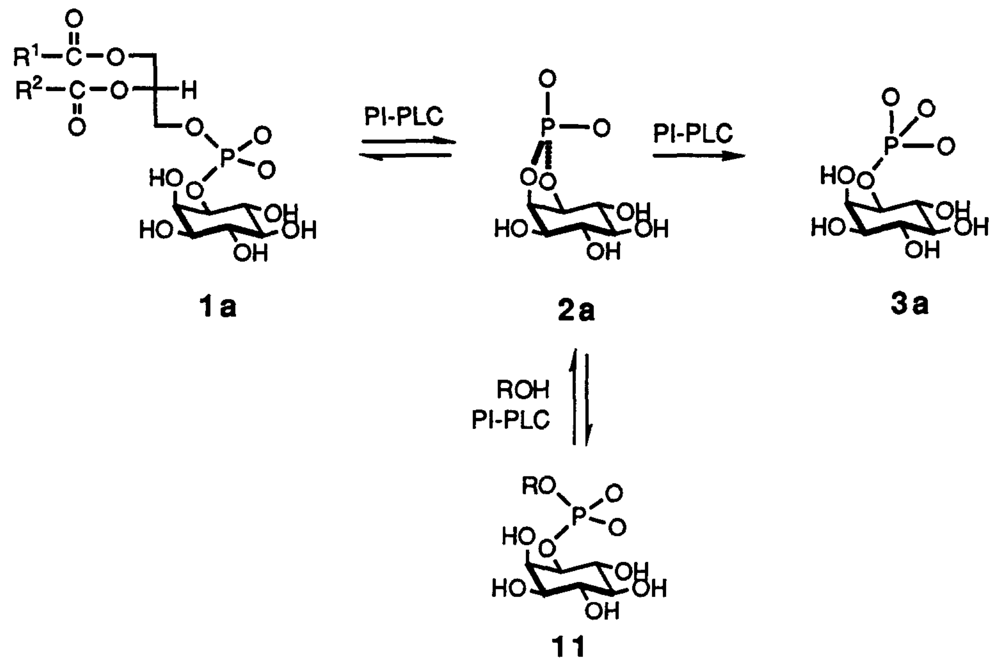


Figure 8. The transesterification reaction catalyzed by PI-PLC.

δ_1 binds strongly to negatively charged PIP_2/PC vesicles ($K_a = 10^6\ M^{-1}$) but not to vesicles composed of only zwitterionic lipids.[139] The activity of PI-PLC-δ_1 decreases with the increase of surface pressure in PIP_2[140] and PI[122,141] monolayers. This result is consistent with a necessity to insert a small part (*ca* 1%) of the protein molecule into a monolayer prior to hydrolysis.[140]

Bacterial PI-PLC display little activity with pure PI as a substrate,[89,90] but are activated when PI is presented to the enzyme as mixed micelles with other phospholipids.[102,143,144] The enzyme from *B. thuringiensis* is activated by short-chain PC by a rate factor of 10^3–10^4.[145] The highest degree of activation by detergents typically occurs at their low concentration (*ca* 0.1% SDC).[59,90,146] However, even the relatively small family of bacterial PI-PLCs is not uniform with regard to the effect of phospholipids and detergents on activity. For example, the *Cytophage* enzyme is deactivated by SDC and is not stimulated by PC,[147] while the *Staphylococcus* enzyme is readily deactivated by nonionic, positively and negatively charged detergents.[89]

(2) Metal ions

Most mammalian PI-PLCs require calcium ions for activity, but not for binding to bilayers.[1,139] The calcium requirement and the concentrations for half-maximal activation are listed in Table 1. In some instances, however, binding of PI-PLC to membranes is calcium dependent (e.g. PI-PLC from chromaffin granules[148] and rat brain[122]). The half-maximal concentrations of calcium are much lower with PIP_2 as a substrate than with PI,[63,79,150] but these parameters vary for different types of PI-PLC from 30 nM[149] to several millimolar.[47,50,59,73] Higher Ca^{2+} concentration could become inhibitory for PIP_2.[15,79,151]

Some PI-PLC-α are only slightly stimulated by calcium ions.[61] A species of PI-PLC from smooth muscle cell is also different from other types in that the hydrolysis of both PIP_2 and PI is stimulated even at millimolar Ca^{2+}.[151] Interestingly, murine B lymphocytes contain another type of PI-PLC specific for PI which is exclusively activated by Mg^{2+}.[152] The 98 kDa PI-PLC from platelet cytosol is activated by both Ca^{2+} and Mg^{2+}.[62] The activation by Mg^{2+} could function as a permanent 'on' switch of PI-PLC in the absence of agonist stimulation when the level of intracellular Ca^{2+} is too low for PI-PLC activation.[62] Many types of mammalian PI-PLC are reversibly inhibited by EDTA, and heavy metals such as La^{3+}, Hg^{2+}, Cd^{2+}, Zn^{2+} and Cu^{2+}.[1,47,149]

Two types of GPI-PLC from rat liver differ in their metal cofactor requirements.[107,109] Both enzymes are membrane-bound; the 52 KDa protein[107] does not require calcium, while the second type (of an unknown molecular weight) is activated by calcium at concentrations slightly higher than physiological.[109] The latter is also inactivated by metal chelating agents. The above properties are shared by two subtypes of membrane-associated GPI-PLC activities in bovine brain, one of which is calcium cation-chelator sensitive and the other one is calcium-independent.[108]

In contrast to mammalian enzymes, bacterial PI-PLCs and trypanosomal GPI-PLCs do not require metal ions for activity,[90] nor are they inactivated by EGTA, EDTA[87,90] or *o*-phenanthroline.[153] The inactivation by some metal ions takes place only at high (submolar) concentrations.[154] These results clearly indicate that metal ions are not cofactors of bacterial PI-PLC.

Assays of PI-PLC Activity

Major assay methods used for phospholipases have been reviewed recently;[155] however, many of the reviewed methods are not applicable to PI-PLC. In particular, titrametric assays have not been used for PI-PLC since bacterial enzymes do not liberate acids during the formation of IcP. Mammalian enzymes liberate acids (acyclic phosphates), but it is not a good measure of the total activity due to simultaneous formation of IcP.

(1) Assays employing radiolabeled substrates

These methods utilize [^{3}H]-labeled PI[156] or [^{3}H]-labeled PIP_2[54] as substrates. ^{32}P-Labeled phosphoinositides[157] are less suitable due to a rapid decay of radioactivity. Polar products are separated from substrates by extraction of assay mixtures with organic solvents. The extent of the reaction is determined from the radioactivity remaining in the organic or aqueous phase after phase separation. Alternatively, the assay mixture is passed through the reversed-phase C_{18} resin to remove the lipid. The water soluble phosphate is then quantified by counting radioactivity after filtering off and washing the resin.[138] Separation can also be achieved by thin-layer chromatography. These methods are laborious and discontinuous, but they are very sensitive and are by far the most commonly used.

(2) Spectrophotometric assays

Dye release. p-Nitrophenyl-1-phosphoinositol (**12**, Figure 9) was used as an assay substrate for *B. cereus* and *B. thuringiensis* PI-PLCs.[158,159] Formation of IcP is accompanied by the release of *p*-nitrophenol which is quantified by UV spectrophotometry. This assay has the advantage of being continuous. However, the unaggregated substrate **12** binds only very weakly to bacterial PI-PLC (K_m > 15 mM).

Thiol assay. Hexadecylthio-1-phosphoinositol (**13**), a phosphorothiolate analog of PI, releases hexadecylthiol upon the cleavage to IcP by *B. cereus* PI-PLC.[160,161] In the presence of 4,4'-dipyridyl disulfide the thiol is converted to mixed pyridylalkyl disulfide thereby producing an equivalent amount of highly chromophoric pyridyl-4-thiol which is then quantified by UV spectrophotometry.[162–165] This assay offers continuity and ensures relatively good interfacial binding of the substrate to the enzyme. However, the V_{max} of **13** is only about 1% of that with natural PI.[161] The corresponding phosphorothiolate analogs of PI (**14**) have been synthesized[166] but the kinetic parameters are comparable to those of **13**.[166b]

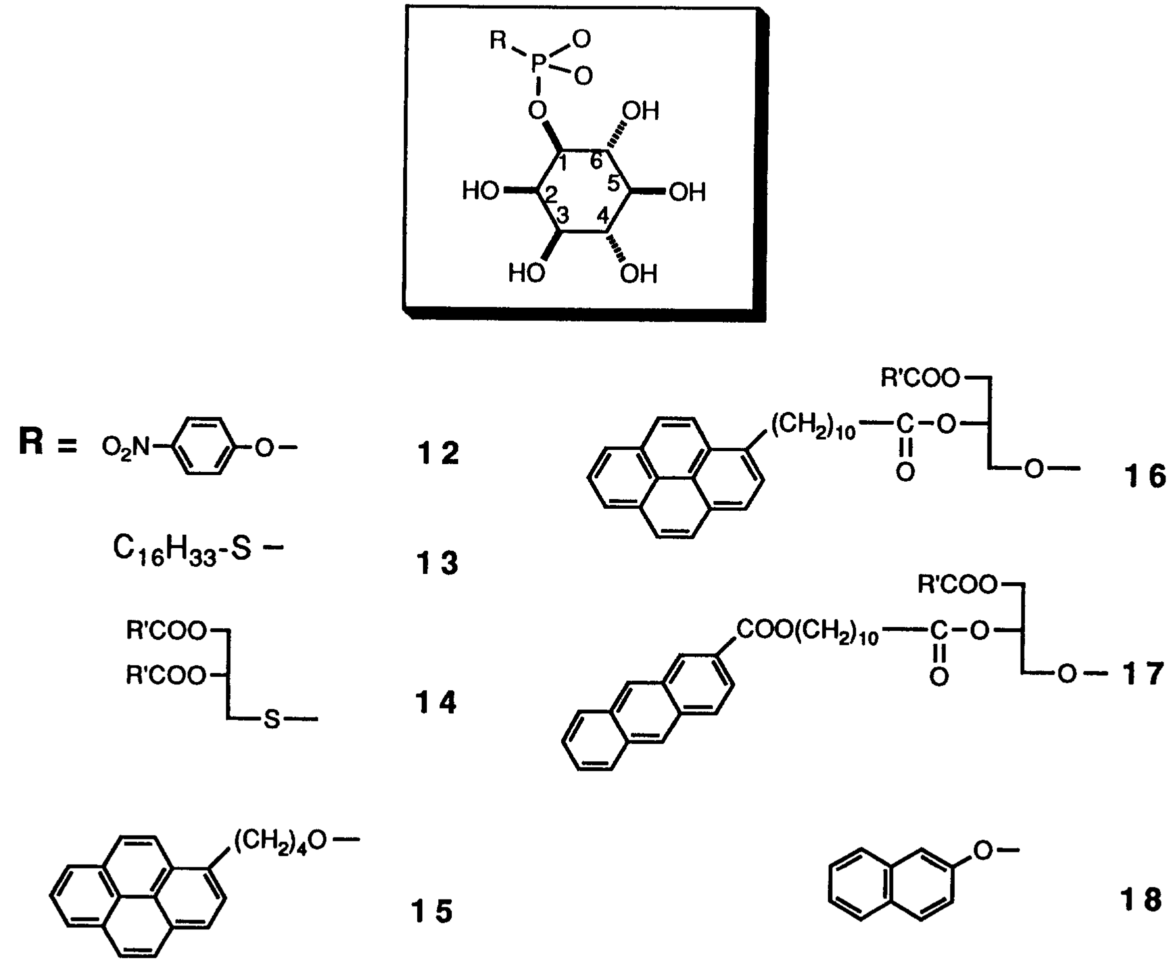


Figure 9. Substrate analogs useful in spectrophotometric assays of PI-PLC.

Application of safranine. Metachromic properties of a dye, safranin, were used as a basis of another PI-PLC assay.[167] Interaction of safranin with negatively charged molecular aggregates brings about a decrease in the absorbance at 520 nm. The change is proportional to charge density at the interface and therefore also to the concentration of PI. This relatively simple assay allows determination of PI-PLC activity at the level of several milliunits.

(3) Coupled enzymatic assay

This method has been used for the determination of inositol phosphates in biological tissues,[168] but it can be adapted for a continuous PI-PLC assay. Inositol phosphate released from PI-PLC reaction is dephosphorylated with alkaline phosphatase to give *myo*-inositol, which is further oxidized to *scyllo*-inosose with inositol dehydrogenase. The unfavorable equilibrium of this process can be shifted by oxidation of NADH by diaphorase, the enzyme that converts nonfluorescent dye resazurin into highly fluorescent resorufin. Resorufin is then continuously determined with a fluorometer.

(4) Fluorometric assays

(A) A discontinuous method utilizing 4-(1-pyrene)butyl-1'-phospho-*myo*-inositol (**15**) as a substrate.[161,169] The substrate upon hydrolysis gives pyrenebutanol which is quantified by fluorometry after it is separated from the mixture by HPLC. This assay is quite laborious, and the activity of *B. cereus* PI-PLC with this substrate analog is only 4% of that with natural substrate. The more promising substrate analogs (**16** and its phosphate derivatives, and **17**) have been obtained by a chemical–enzymatic method,[170,171] but their use in kinetic studies has not yet been reported. (B) A continuous fluorometric assay utilizing β-naphthyl-1-phospho-*myo*-inositol (**18**).[172] The measurement is based on the batochromic shift of the emission maximum of product β-naphthol as compared to the substrate phosphodiester. A severe disadvantage of this method is the extremely slow turnover (0.003% of the rate with PI) of the fluorescent substrate.[172]

(5) Monolayer assay

The radiolabeled substrate such as PI or PIP_2 is spread as a lipid monolayer at the water–air interface followed by the addition of the enzyme preparation to the subphase. The progress of the reaction is monitored by means of a Geiger–Mueller tube suspended just above the monolayer[122,139,173] or by counting water soluble phosphates in the subphase.[139] Using the barostat technique and the so-called zero-order Langmuir trough, it is possible to use the unlabeled phospholipids as substrates.[173] The contraction of the monolayer area as a function of time is a measure of the reaction rate. Monolayer assays offer many advantages over the bulk substrate assays among which minimization of the enzyme and substrate usage, and mimicking the conditions existing in the natural membrane are the most important.[174] As a disadvantage, partial loss of activity has been reported with some enzymes due to surface denaturation or adsorption into the hydrophobic trough walls.[139,174]

(6) Release of GPI-anchored proteins

Rat kidney tissue or bovine erythrocytes are incubated with PI-PLC and the released alkaline phosphatase (AP) or acetylcholinesterase (AChE), respectively, are quantified using chromophore-producing substrates such as *p*-nitrophenyl phosphate and acetylthiocholine.[87] Both methods are discontinuous.

(7) Phosphorus-31 NMR

^{31}P NMR is probably the most versatile method, allowing for simultaneous quantification and identification of hydrolytic products of PI-PLC.[93,94,123,124,175,176] The distinction between the substrate, the cyclic and the acyclic inositol phosphates is easily made due to the low-field chemical shift of the cyclic phosphates (*ca* 16 ppm for IcP, 4 ppm for IP, -1.5 ppm for PI). The best utility of this method is in semiquantitative measurements and in verification of the structure of products. In order to obtain spectra with sufficiently narrow line-shapes monomeric or micellar substrates have to be used, with detergent–phospholipid ratios substantially higher than those typically recommended for maximal activity.

(8) Mass spectrometry

This technique is used more frequently for structural assignments than for absolute product quantification. Quantification of nonvolatile inositol phosphates requires prior derivatization into persilyl or perfluoroacyl derivatives.[177] Determination of the ratio of cyclic/acyclic inositol phosphate can be achieved by acidic hydrolysis of cyclic phosphates in $H_2{}^{18}O$ followed by removal of the phosphate with alkaline phosphatase, conversion of the phosphate into its tris(*tert*-butyldimethylsilyl) ester and measurement of ^{18}O enrichment by mass spectrometry.[64]

(9) Miscellaneous assays

A number of useful methods of quantification of inositol phospholipids and inositol phosphates derived therefrom is described in the collective work *"Methods in Inositide Research"*.[179] A large effort has been devoted to developing alternative methods for detection of inositol phosphates (other than radioassay). HPLC chromatography in conjunction with the post-column complexometry[180,181] or ion chromatography with conductometric detection[182] are among the best choices.

Substrate Analogs and Inhibitors

Isozyme-specific inhibitors of PI-PLCs are of enormous therapeutic potential, especially as anti-inflammatory agents.[81,183] They may also be important tools in establishing the correlations between various branches of phosphoinositide metabolism and in elucidation of the mechanism and structure of PI-PLCs. However, most of the synthetic activity in the area of phosphoinositides has been devoted to analogs of inositol polyphosphates rather than phosphatidylinositides (PI and its derivatives).[184,185,186] Inhibition of inositol 1-phosphate phosphatase,[185] phosphatidylinositol kinases,[183,187,188] and IP_3-receptor binding[189] have attracted more attention.

To date the inhibition studies of PI-PLC have not tried to resolve the interfacial binding and active site binding steps. Furthermore, various forms of PI-PLC (α–ε) have different origins (cytosolic or membrane bound) and thus probably different interfacial binding properties. It is thus important that the enzyme preparations tested be resolved into subtype species. Unfortunately this is not the case in many of the works cited below. For these reasons the substrate specificity and inhibition properties described in the following sections should be interpreted with great caution.

(1) Modification of the diacylglycerol moiety

The diacylglycerol moiety appears not to be an important determinant for catalysis, but it may be important for the partition of substrates into micelles or bilayers. Neither mammalian nor bacterial PI-PLCs discriminate between compounds having L or D configurations at the glycerol C-2 carbon.[94] Lysophosphatidylinositol,[85] other single chain primary alkyl,[169] alkylthio[160,161] and aryl[158,159,172] esters of inositol 1-phosphate are also cleaved, though at reduced rates. The deacylated glycerophosphoinositol is hydrolyzed by *B. thuringiensis* PI-PLC at a rate lower than that of IcP.[132] The preference for a certain type of fatty acids is only several fold.[190] The deacylated analog of PIP is an inhibitor of PI-PLC,[81] whereas single chain alkyl phosphoinositol analogs (in which the diacylglycerol is replaced by a long chain alcohol) inhibit guinea pig PI-PLC-α with IC_{50} = 14–50 μM.[188]

(2) Modification of the phosphodiester moiety

The phosphodiester moiety is important for catalysis. The phosphorothioate analogs, (R_p)- and (S_p)-DPPsI (**6**), show substantially decreased activities. In addition, both mammalian and bacterial PI-PLCs display a high degree of stereoselectivity toward the R_p isomer.[123–125] A series of phosphonate sulfonate or methyleneoxy analogs of PI, (**19–22**, Figure 10) have been synthesized,[206–209] but no follow up study on PI-PLC inhibition with these analogs has been reported. The related single chain phosphonate analogs **23** were found to be weak inhibitors of PI-PLC from *B. cereus* with IC_{50} in the range 4 to 7 mM.[192,193]

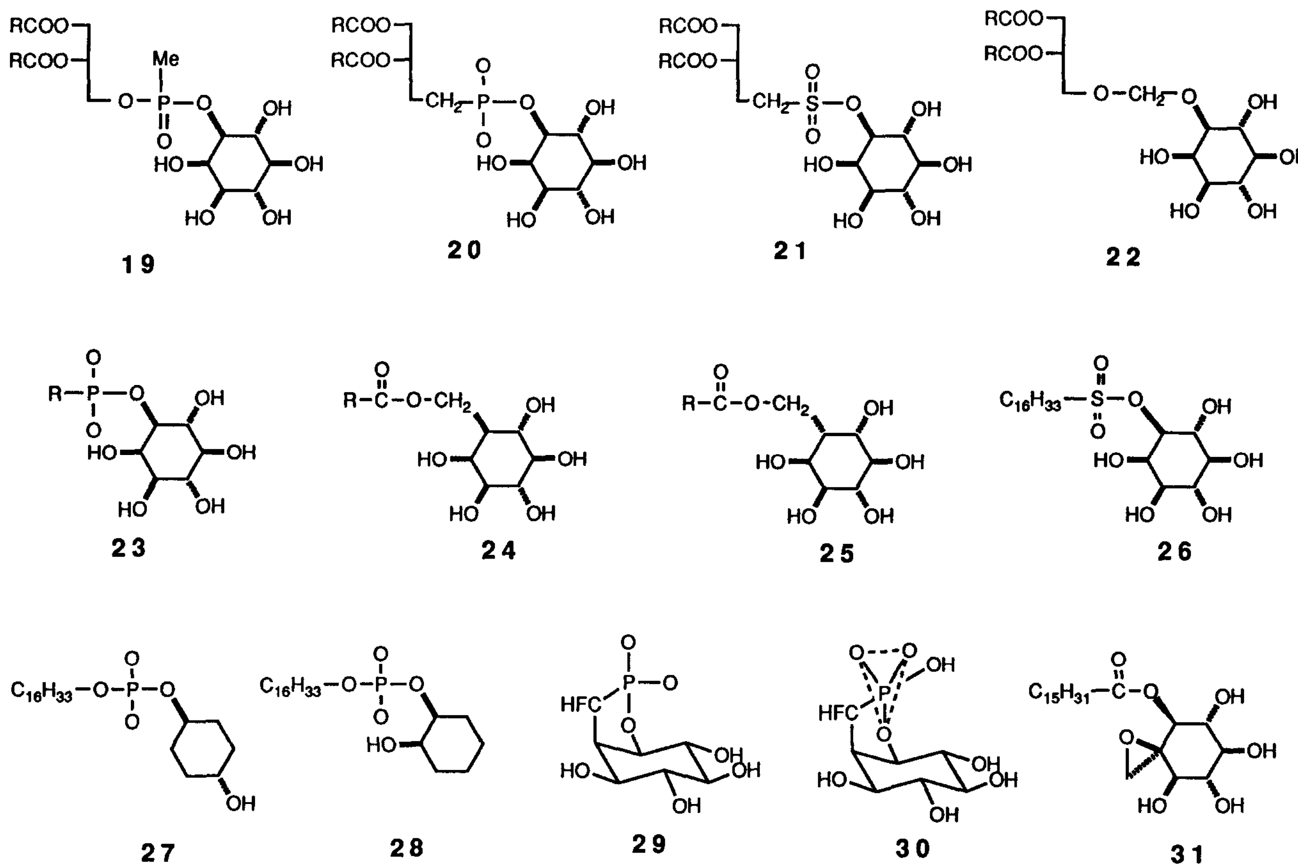


Figure 10. Structures of some of the inhibitors of PI-PLC. Structure **30** is an analog of a possible transition state for the hydrolysis of IcP to IP.

The inhibitory properties of the analogs in which the phosphodiester group is replaced by nonionic groups vary. Carbamate derivatives of *myo*-inositol,[204,205] palmitoyl derivatives of *myo*-inositol[191], and palmitoyloxymethylene derivatives of *myo*- and *chiro*-inositol (**24** and **25**, respectively)[191,197] are all poor inhibitors; however, introduction of phosphates or sulfates at the inositol ring increases the inhibitory capability of some of these analogs to crude platelet PI-PLC, with IC_{50} in the range of 10 to 100 μM.[191] On the other hand, the PI-PLC-α from guinea pig is inhibited, with IC_{50} in the same range, by non-phosphorylated palmitoylinositol, palmitoylinositol, and the alkylsulfonyl derivative of inositol (**26**).[188]

(3) Modification of the inositol moiety

Interactions with the inositol moiety of PI are important for recognition. Both substrate and inhibitor activities are affected by such changes as inositol phosphorylation, substitution of hydroxyl groups, deoxygenation and configurational changes of inositol. The results are summarized as follows: (i) The 1D-configuration of *myo*-inositol is absolutely required for bacterial PI-PLC.[93,159,172] 1D-Inositol-1,4,5-trisphosphate was found to inhibit binding of PI-PLC-δ_1 to lipid bilayers while the corresponding enantiomer showed no effect;[194] (ii) according to our proposed sequential mechanism[94] the axial hydroxyl group at the 2-position of inositol should be absolutely required for catalysis. Supporting this view is the fact that 2-deoxy-PI is not a substrate of human melanoma PI-PLC[198] and bovine brain PI-PLC-β_1, -γ_1 and δ_1.[199,200] It is only a weak inhibitor of the melanoma enzyme (IC_{50} > 2 mM) implying that the 2-OH group is also important for overall binding.[198] Likewise, GPI species acylated with fatty acid at 2-position are resistant to PI-PLC from *S. aureus*;[99-101,201] (iii) The orientation of the 3-OH group is an important determinant for the substrate, since inversion of the 3-OH group of the natural 1D-*myo*-PI (the resulting analog is 1L-*chiro*-PI, see Figure 12 for structures and numbering) causes a 10^3-fold decrease in the activity towards bacterial PI-PLCs.[196] The fact that its enantiomer, 1D-*chiro*-PI, shows no detectable activity (<10^{-6} relative to 1D-*myo*-PI) is consistent with the requirement of 1D-configuration for *myo*-PI mentioned in (i). This result, however, contradicts an earlier report that PI-PLC from *S. aureus* cleaves GPI-anchor containing D-*chiro*-inositol;[97,98,195] (iv) in agreement with the importance of the 3-OH group, phosphorylation at the 3-position makes PI resistant to mammalian enzymes;[202,203] (v) although the structural requirement for catalysis is stringent at the inositol ring, that for binding or inhibition is not. The 2,3,5,6-tetradeoxy analog of palmityl PI (**27**) inhibits guinea pig PI-PLC-α with an IC_{50} of 25 μM; however, the corresponding 3,4,5,6-tetradeoxy analog (**28**) is a poor inhibitor.[188] These results suggest that deoxygenation can be tolerated in binding to PI-PLC-α, except at the 4-position.

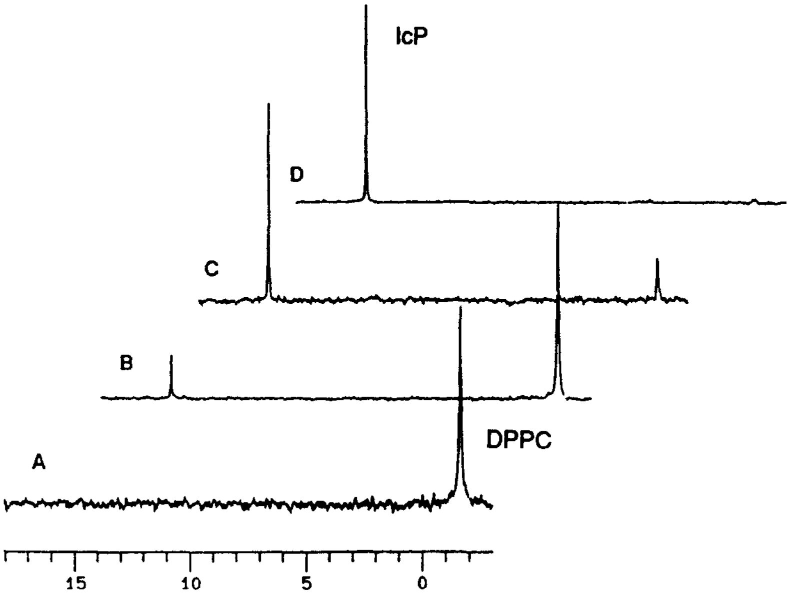


Figure 11. Inactivation of PI-PLC (*B. thuringiensis*) by the epoxide analog **31** monitored by ^{31}P NMR. (A) 2.5 μmol of DPPI in 0.4 ml of 0.1 M Tris·HCl, pH 7.0, containing 0.1 M sodium deoxycholate; (B) 90 min after addition of 3.5 μg of PI-PLC preincubated with 0.4 mM of the epoxide analog; (C) same as B, after 12 h; (D) control experiment in the absence of the inhibitor, 5 min after addition of PI-PLC. The remaining enzyme activity in B-C is <1% relative to that in D.

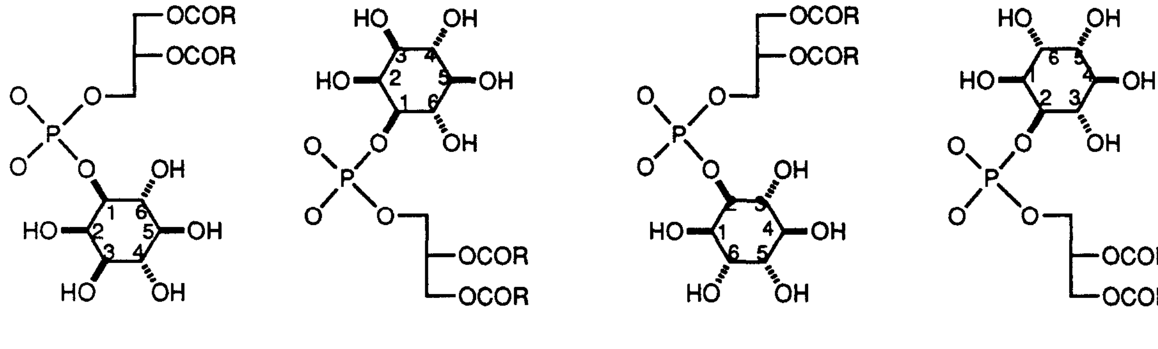


Figure 12. Structures of 1D-*myo*-PI (natural substrate for PI-PLC), 1L-*myo*-PI (no detectable activity), 1L-*chiro*-PI (the 3-epimer of 1D-*myo*-PI, poor substrate), and 1D-*chiro*-PI (no detectable activity). The numbering conventions are different between the derivatives of *myo*- and *chiro*-inositols according to 1976 IUPAC rules [*Biochem. J.* **153**, 23–31 (1976)].

(4) Analogs of IcP

The fluorophosphonate analogue of IcP **29** was found to be a modest inhibitor of *B. cereus* PI-PLC.[210] According to recent calculations[154] the fluoromethylene group prefers an apical position in the phosphorus trigonal bipyramid of a transition state or an intermediate (**30**) in a substitution reaction; therefore fluoromethylene group in IcP analog should stabilize a trigonal bipyramid intermediate (or a transition state).

(5) Epoxide analogs

In an attempt to develop active site directed irreversible inhibitors we have synthesized an epoxide analog of palmitoyl PI, **31**. Despite the lack of the phosphatidyl moiety, this analog inhibits *B. thuringiensis* PI-PLC as shown in Figure 11 (Bruzik and Tsai, unpublished results). Although preliminary, such results suggest that epoxy analogs of PI and its derivatives are likely to be good inactivators of PI-PLC.

(6) Nonspecific inhibitors

Most of the inhibitors described in this section either interact with the enzymes nonspecifically or do not interact with the enzymes directly. For the latter group, the term 'inhibitor' is used in a broad sense. Phenothiazine-type drugs including methyldiazine, promethazine, chlorpromazine, chloroquine, quinacrine and Cibacron Blue[77,205,211] are inhibitors of PI-PLC with IC_{50} in the range 3 to 300 μM. Various aminoglycoside antibiotics are also good inhibitors of PI-PLC.[212] The potency of streptomycin, amikacin, kanamycin, tobramycin, gentamycin and neomycin as inhibitors of renal PI-PLC correlates positively with their nephrotoxicity (basicity) with IC_{50} in the range 30 to 380 μM.[212] The inhibition presumably arises from the ionic interaction between positively charged protonated aminoglycoside and the negatively charged phospholipid aggregates. Consistently, neomycin is a stronger inhibitor of the hydrolysis of PIP_2 than of PIP.[213] The inhibition of human platelet PI-PLC by aliphatic alkanediamines[214] and polyamines[216] is probably based on the same mechanism. The effect of polyamines and aminoglycoside on the activity of human amnion PI-PLC appears to be dependent on the concentration of divalent metal ions. Amines are inhibitory at low concentrations of Ca^{2+}, but are stimulatory at high Ca^{2+}.[215] These complex dependencies probably result from a competitive interaction of divalent metal ions and protonated amino-compounds with negatively charged phospholipid bilayers.

A diether analog of PC with stearyl ether at *sn*-1 and methyl ether at *sn*-2 is one of the most potent inhibitors of fibroblast and adenocarcinoma cell PI-PLC (IC_{50} = 0.4 μM).[217] Recently a polycyclic xanthone derivative, vinaxanthone, has been found to have a strong inhibitory effect on rat brain PI-PLC with IC_{50} at the level 5.4 μM.[218] The mechanism of the inhibition by these two compounds remains unclear. Manoalide, a terpenoid shown earlier to inhibit phospholipase A_2,[264] is also inhibitory towards PI-PLC from guinea pig uterus.[219] The inhibition is irreversible and time-dependent with IC_{50} 3-6 μM, and occurs due most likely to covalent modification of the enzyme. Treatment of platelet PI-PLC with thiol-specific reagents such as 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) or methyl methanethiosulfonate strongly inhibits its activity.[220]

Syntheses of Substrates and Substrate Analogs

Although the synthesis of inositol phosphates and their analogs has been well developed as reviewed recently,[184–186] synthesis of phosphatidylinositides such as PI, PIP, PIP_2 and their analogs is a much more challenging task due to the need not only to protect the hydroxyl groups, but also to protect them differentially to allow introduction of the phosphatidate and the phosphates. Moreover, the presence of the alkali-labile diacylglycerol precludes application of the acyl-protecting groups. In addition, certain analogs of phosphatidylinositols (e.g. those containing unsaturated fatty acids or phosphorothioates) preclude the use of hydrogenolytically removable protective groups. Therefore most of the enzymatic work has been performed using naturally occurring PI, PIP and PIP_2. Early synthetic efforts were summarized by Shvets[221,222] and Gigg.[223] Recently, total chemical syntheses of PI[94,187,224–226,231] PIP,[226] PIP_2,[227,228] and *chiro*-PI[196] have been described. PI[190,229,230], PIP[230] and some analogs with altered fatty acids[170,171,229] have also been obtained semisynthetically using naturally available phosphatidylinositols.

The synthetic work summarized above involves a variety of approaches for the synthesis of protected *myo*-inositol precursors. Many of the precursors are aimed at a specific target compound. Furthermore, most of the procedures involve numerous protecting and optical resolution steps, which lead to low overall yield. To overcome such problems, we have developed a systematic and efficient route for the synthesis of enantiomerically pure and regiospecifically protected *myo*-inositols.[265] The key strategies are the use of camphor as a protecting group *and* a chiral auxiliary, and the development of regiospecific controls in various steps. These precursors can in principle lead to most, if not all, of the naturally occurring phosphatidylinositols, their phosphate derivatives, and inositol phosphates. The procedures can also be modified to synthesize various unnatural analogs and inhibitors.

Sequences and Structures

The sequences of many bacterial and mammalian PI-PLCs have been reported as summarized in Table 2. However, except for the secondary structure of PI-PLC-α,[61] no structural study of bacterial or mammalian enzymes has been reported to date. Mammalian PI-PLCs are difficult to obtain in a quantity large enough for crystallization study; the instability of many PI-PLC isoforms over a longer period of time may also be a problem. The Oregon group has succeeded in crystallization of PI-PLC from *B. cereus*. Crystals of two types with hexagonal and orthorhombic packing were obtained; the latter diffracted to 2.5 Å.[232] However, the molecular structure remains to be solved. In the absence of tertiary structures, sequence analysis is important in understanding the structure–function relationship of PI-PLC.

(1) Mammalian PI-PLC

Analysis of sequences of mammalian PI-PLCs now available (Table 2) indicates that although all PI- and GPI-PLC catalyze the cleavage of the phosphodiester bond in structurally closely related substrates, only a limited sequence homology exists between these proteins.[4,9,11,13,233] Molecular sizes of PI-PLC range from 17 kDa for PI-PLC from human spleen to 150 kDa for PI-PLC-β_3 from bovine brain. Based on the molecular size, sequence, and immunological cross-reactivity, mammalian PI-PLCs have been divided into several categories:[4] PI-PLC-α (MW 56–68 KDa)[10,58,234–236] PI-PLC-β (150–154 kDa),[47,48,233,237,238] PI-PLC-γ (145–148 kDa),[45,47,48,238,240–242] PI-PLC-δ (85–88 kDa)[46,239,243,244] and PI-PLC-ε (86 kDa).[245] Each β, γ and δ category of mammalian PI-PLC is further divided into several related subcategories differentiated by the subscripts such as β_1, β_2, β_3, γ_1, γ_2, δ_1, δ_2 and δ_3. The enzymes from β, γ and δ categories differ in the mechanism of regulation and do not cross react with antibodies raised against species from another category. The overall sequence homology between the β -, γ- and δ-PI-PLC is also low. On the other hand, the enzymes belonging to the same category show immuno-crossreactivity, are regulated by the same mechanism and share high sequence homology. The differences between the enzymes of the same subcategory from various tissues, animal genuses or species are minimal. As an example, rat, bovine and human brain PI-PLC-γ_1 show more than 95% sequence homology.[11]

Table 2. Published sequences of PI-PLC

Source	Type	Other names	Calculated FW (number of amino acids)	Accession Number in EMBL/GenBank	Reference
B. cereus			34,466 (298, mature)	M28549	42
B. thuringiensis			(330, precursor)	X12952	259
Listeria monocytogenes			36,299 (317, precursor)		246
T. brucei		VSG lipase	40,760 (358)	J04124	103
T. brucei		VSG lipase	40,660 (358)	X13292	104
Guine pig uterus	α	PI-PLC-I	56,559 (504)		235
Rat brain	β_1	PI-PLC-I	138,225 (1216)		239
Bovine brain	β_1	PLC-154	138,600 (1225)	J03137	52
Human promyeolocyte	β_2		(1181)	M95678	15
Human fibroblast	β_3			Z16411	15
rat thyroid cells	β_3		150,000 (1217)	M99567	233
Drosophila	β	norp*A*	(1095)	J03138	260
Drosophila (2 seqs.)	β	PLC-21	1305 (1320)	M60452 (M60453)	261
Rat brain	γ_1	PI-PLC-II	148,431 (1289)	J03806	262
Bovine brain	γ_1	PLC-148	148,300 (1291)	Y00301	242
Human fibroblast	γ_1			M34667	15
Human leukocyte	γ_2	PI-PLC-II	(1252)		240
Human promyelocyte	γ_2			M37238	15
Rat muscle	γ_2	PI-PLC-IV	(1265)	J05155	241
Rat brain	δ_1	PI-PLC-III	85,840 (756)		239
Bovine brain	δ_2		86,941 (764)		244
Dictyostelium discoideum	δ		91,000 (801)	M95783	263

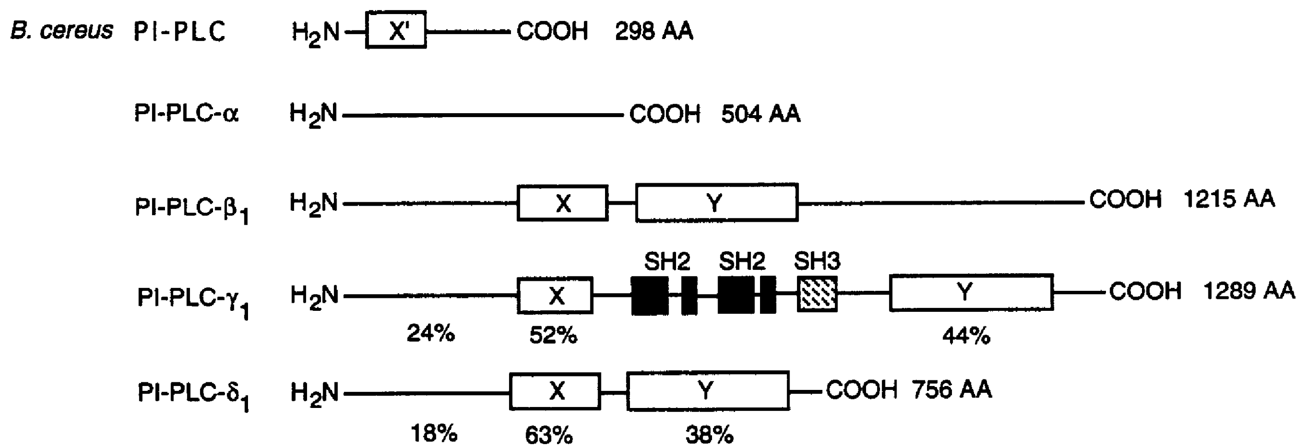


Figure 13. Diagrammatic representation of sequences of various PI-PLC. Homologous polypeptide stretches are shown as X and Y boxes giving degrees of sequence identity with the analogous domains of PI-PLC-β_1 (from ref. 8 with permission).

The low overall homology between the proteins belonging to different categories (β-δ) increases up to 60% and 40% in the two domains designated as X (*ca* 170 amino acids) and Y (*ca* 240 amino acids), respectively[4,8,11–13] (Figure 13). Significant sequence homology outside regions X and Y exists only within the same category. For instance, β_1 and β_2 isozymes exhibit 54% identity in the amino-terminal region (before X box) and 31% identity in C-terminal fragment (after Y box). The X domains in 16 eucaryotic PI-PLCs contain the following stretch of absolutely conserved amino acids: Met–GlnProLeu–HisTyr–Ile–SerSerHisAsnThrTyrLeu---Gln----SerSer–Glu–Tyr---Leu--GlyCysArgCysValGlu LeuAsp–Trp–Gly; the Y domains contain LeuSerArgIleTyrPro–Gly–Arg–AspSerSerAsnTyr–Pro---Trp–Gly–GlnMetValAlaLeuAsn-PheGlnThr (dashes mean nonconserved amino acids). It is likely that these amino acids play important roles in catalysis.

The α-form of bovine PI-PLC, which is similar to bacterial enzymes in terms of metal ion requirement (can catalyze turnover without Ca^{2+}, albeit at a lower rate), does not contain X or Y domain and has no sequence homology with other mammalian species.[13,235] The relationship of PI-PLC-α to other isozymes is even less clear in view of the fact that cDNA cloned is highly homologous to a number of unrelated proteins[58] and does not code for a functional PI-PLC.[12]

There are other types of mammalian PI-PLCs which do not fall into the α–δ categories, such as a 157 kDa protein from rat smooth muscle,[151] a 150 kDa protein from human melanoma cells,[73] high molecular weight PI-PLC from platelets,[56] a 17 kDa protein from human spleen,[74] an 11 kDa PLC activity from platelets,[49] and a Mg^{2+}-dependent PI-PLC.[113] It is possible that the smaller proteins are proteolytic fragments of other PI-PLCs, but no direct evidence has been reported.

(2) Bacterial PI-PLC and trypanosomal GPI-PLC

The molecular weights of bacterial enzymes are *ca* 35 kDa. The sequences of PI-PLCs from *B. cereus* and *B. thuringiensis* differ by only eight amino acids.[42] PI-PLC from *B. cereus* and trypanosomal GPI-PLC, though catalyzing different reactions, are similar in size and highly homologous in the N-terminal region (37%) and in a small central core (50%).[42] Another bacterial enzyme from *Listeria monocytogenes* is also very similar in size and is 24% homologous to *B. cereus* and trypanosomal enzymes.[246] A relatively high degree of homology (26% identity, 52% conservative) exists between bacterial enzymes and segments X of eucaryotic PI-PLCs in positions which are also conserved between eucaryotic enzymes.[42] It is therefore anticipated that these peptide fragments contain elements of the active site.

(3) Active site

Binding experiments of tryptic fragments from *B. cereus* PI-PLC to monoclonal antibodies raised against the intact protein suggest that the active site is located in the peptide stretch between Gln-45 and Lys-122.[247] This tryptic fragment, which contains 9 positively and 9 negatively charged residues, also displays the highest homology to the X-domain of eucaryotic PI-PLCs (Kuppe *et al.* 1990) and is probably important functionally. The possible candidates for active site residues in *B. cereus* PI-PLC include Gln-45, Glu-52, Arg-56, Arg-64, Asp-67 or Glu-67, His-83 and Glu-93 or Asp-93 which are conserved among *B. cereus, B. thuringiensis, T. brucei, Drosophila norp A*, rat β1 and δ1, and bovine γ1 PI-PLCs.[42] Sequence deletion study of PI-PLC-γ_2 showed that a large segment of the protein (up to 1/3 of the total length) residing between the X and Y boxes can be removed with only a small effect on the activity (< 10-fold decrease) while deletions within these boxes results in a complete loss of activity.[241] It was therefore concluded that both X and Y boxes contribute to the structure of the active site, and that the median fragment which has homology to regulatory domains of tyrosine kinases is probably only important for regulation. Analogously, C-terminal or N-terminal truncation of PI-PLC-γ_1 completely abolishes activity but the removal of the regulatory domain results only in a threefold drop of activity.[45] Limited proteolysis of PI-PLC-δ_1 which removes the 60 amino acid N-terminal segment affords a 77 kDa peptide which retains 10% activity of the native protein but has a lower binding affinity to PIP_2

vesicles.[248] The protein remains active even after further cleavage into a heterodimer of 45 kDa and 32 kDa. Therefore, it was postulated that the surface binding site resides in the N-terminal part of PI-PLC-δ. Deletion of a 45 kDa C-terminal fragment of PI-PLC-β_1 affords a fully catalytically active 100 kDa polypeptide.[249] The C terminus is therefore not necessary for catalysis.

Concluding Remarks

Although a vast amount of information has been summarized in this review, it is clear that our understanding of the mechanisms of PI-PLCs and GPI-PLCs is still at the infant stage. No tertiary structure is yet available, a standard and accurate assay method is yet to be developed, and more substrate analogs and inhibitors are yet to be developed. However, it is also clear that significant progress has been made in each of these areas, and that major developments are imminent.

Acknowledgements

The authors would like to thank Dr. D. Baker, M. Bergers, P. Buetikofer, U. Brodbeck, G. Carpenter, H. Chap, S. Cockroft, E. A. Dennis, P. T. Englund, J. H. Exton, J. N. Fain, H. Goldfine, O. H. Griffith, T. K. Harden, H. S. Hendrickson, H. Ikezawa, R. Kriz, M. D. Mamrack, Y. Nozawa, M. J. Rebecchi, S.-G. Rhee, T. L. Rosenberry, T. Takenawa, P. Wyss, and R. C. Young for providing reprints of their publications or sharing their results prior to publication.

References

1. Shukla, S. D. *Life Sci.* **1982**, *30*, 1323–1335.

2. Crooke, S. T. *Cell Calcium* **1989**, *10*, 309–23.

3. Majerus, P. W.; Connolly, T. M.; Deckmyn, H.; Ross, T. S.; Bross, T. E.; Ishii, H.; Bansal, V. S.; Wilson, D. B. *Science*, **1986**, *234*, 1519–1526.

4. Rhee, S. G.; Suh, P. G.; Ryu, S. H.; Lee, S. Y. *Science* **1989**, *244*, 546–550.

5. Bansal, V. S.; Majerus, P. W. *Ann. Rev. Cell. Biol.* **1990**, *6*, 41–67.

6. Deckmyn, H.; Whiteley, B. J.; Majerus, P. W. In *Mol. Pharmacol. Cell Regul.* 1, *(G-Proteins Mediators Cell. Signalling Processes* pp. 429–452, Iyengar, R.; Birnbaumer, L. Eds., Academic Press, San Diego, 1990.

7. Litosch, I. In *Mol. Pharmacol. Cell Regul.* 1, *(G-Proteins Mediators Cell. Signalling Processes*, pp. 453–476, Iyengar, R.; Birnbaumer, L. Eds., Academic Press, San Diego, 1990.

8. Dennis, E. A.; Rhee, S. G.; Billah, M. M.; Hannun, Y. A. *FASEB J.* **1991**, *5*, 2068–2077.

9. Meldrum, E.; Parker, P. J.; Carozzi, A. *Biochim. Biophys. Acta* **1991**, *1092*, 49–71

10. Mobbs, C. V.; Kaplitt, M.; Kow, L.-M.; Pfaff, D. W. *Mol. Cell. Endocrin.* **1991**, *80*, C187–191

11. Rhee, S. G. *Trends Biochem. Sci.* **1991**, *16*, 297–301.

12. Rhee, S. G.; Choi, K. D. *J. Biol. Chem.* **1992**, 267, 12393–12396.

13. Rhee, S. G.; Choi, K. D. *Adv. Second Messenger Phosphoprotein Res.* **1992**, *26*, 35–60.

14. Sternweis, P. C.; Smrcka, A. V. *Trends Biochem. Sci.* **1992**, *17*, 502–505.

15. Kriz, R.; Lin, L.-L.; Sultzman, L.; Ellis, C.; Heldin, C.-H; Pawson, T.; Knopf, J. *Proto-oncogenes In Cell Development*, CIBA Foundation Symp. **1990**, *150*, 112–127.

16. Harden T. K. *Adv. Second Messenger Phosphoprotein Res.* **1992**, *26*, 11–34.

17. Cockroft, S.; Geny, B.; Thomas, G. M. H. *Biochem. Soc. Trans* **1991**, *19*, 299–302.

18. Cockroft, S.; Thomas, G. M. H. *Biochem. J.* **1992**, *288*, 1–14.

19. Jones, G.; Carpenter, G. *Progr. Growth Factor Res.* **1992**, *4*, 97–106.

20. Fain, J. N. *Biochim. Biophys. Acta* **1990**, *1053*, 81–88.

21. Wahl, M.; Carpenter, G. *Bioassays* **1991**, *13*, 107–113.

22. Low, M. G. *Biochem. J.* **1987**, *244*, 1–13.

23. Low. M. G.; Saltiel, A. R. *Science* **1987**, *239*, 268–275.

24. Ferguson, M. A. J.; Williams, A. F. *Ann. Rev. Biochem.* **1988**, *57*, 285–320.

25. Homans, S. W.; Ferguson, M. A. J., Dwek, R. A.; Rademacher, T. W.; Anand, R.; Williams, A. F. *Nature* **1988**, *333*, 269–272.

26. Low, M. G. *FASEB. J.* **1989**, *3*, 1600–1608.

27. Cross, G. A. M. *Ann. Rev. Cell Biol.* **1990**, *6*, 1–39.

28. Doering, T. L.; Masterson, W. J.; Hart, G. W.; Englund, P. T. *J. Biol. Chem.* **1990**, *265*, 611–614.

29. Thomas, J. R.; Dwek, R. A.; Rademacher, T. W. *Biochemistry* **1990**, *29*, 5413–5422.

30. Ikezawa, H. *Cell Biol. Int. Rep.* **1991**, *15*, 1115–1131.

31. Ferguson, M. A. J. *Biochem. Soc. Trans.* **1992**, *243*, 243–256.

32. Carrington, M.; Walters, D.; Webb, H. *Cell Biol. Int. Rep.* **1991**, *15*, 1101–1113.

33. Field, M. C; Menon, A. K. In *Lipid Modifications of Proteins* pp. 83–135, Schlesinger, M. J. Ed. CRC Press, Boca Raton, 1993.

34. Englund, P. T. *Annu. Rev. Biochem.* **1993**, *62*, 121–138.

35. Houslay, M. D.; Wakelam, M. J. O.; Pyne, N. J. *Trends Biochem. Sci.* **1986**, *11*, 393–394.

36. Saltiel, A. R.; Fox, J. A.; Sherline, P.; Cuatrecasas, P. *Science* **1986**, *233*, 967–972.

37. Czech, M. P.; Klarlund, J. K.; Yagaloff, K. A.; Bradford, A. P.; Lewis, R. E. *J. Biol. Chem.* **1988**, *263*, 11017–11020.

38. Romero, G.; Luttrell, L.; Rogol, A.; Zeller, K.; Hewlett, E. Larner, J. *Science* **1988**, *240*, 509–511.

39. Romero, G. *Cell Biol. Int. Rep.* **1991**, *15*, 827–852.

40. Saltiel, A. R.; Cuatrecasas, P. *Am. J. Physiol.* **1988**, *255*, C1–C11.

41. Hough, E.; Hansen, L. K.; Birkness, B.; Jynge, K.; Hansen, S.; Hordvik, A., Little, C.; Dodson, E.; Derewenda, Z. *Nature* **1989**, *338*, 357–360.

42. Kuppe, A.; Evans, L. M.; McMillen, D. A.; Griffith, O. H. *J. Bacteriol.* **1989**, *171*, 6077–6083.

43. Lenstra, R.; Mauco, G.; Chap, H.; Douste-Blazy, L. *Biochim. Biophys. Acta* **1984**, *792*, 199–206.

44. Baldassare, J. J.; Henderson, P. A.; Fisher, G. J. *Biochemistry* **1989**, *28*, 6010–6016.

45. Bristol, A.; Hall, S. M.; Kriz, R. W.; Stahl, M. L.; Fan, Y. S.; Byers, M. G.; Eddy, R. L.; Shows, T. B.; Knopf, J. L. *Cold Spring Harbor Symp. Quant. Biol.* **1988**, *53*, 915–920.

46. Rebecchi, M. J.; Rosen, O. M. *J. Biol. Chem.* **1987**, *262*, 12526–12532.

47. Ryu, S. H.; Cho, K. S.; Lee, K.-Y.; Suh, P.-G.; Rhee; S. G. *J. Biol. Chem.* **1987**, *262*, 12511–12518.

48. Ryu, S. H.; Suh, P. G.; Cho, K. S.; Lee, K. Y.; Rhee, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 6649–6653.

49. Carter, H. R.; Smith, A. D. *Biochem. J.* **1987**, *244*, 639–645.

50. Homma, Y.; Imaki, J.; Nakanishi, O.; Takenawa, T. *J. Biol. Chem.* **1988**, *263*, 6592–6598.

51. Carter, H. R.; Wallace, M. A.; Fain, J. N. *Biochim. Biophys. Acta* **1990**, *1054*, 119–128.

52. Katan, M.; Kriz, R. W.; Totty, N.; Philp, R.; Meldrum, E.; Aldape, R. A.; Knopf, J.; Parker, P. J. *Cold Spring Harbor Symp. Quant. Biol.* **1988**, *53* (*Mol. Biol. Signal Transduction, Part 2*), 921–926.

53. Rhee, S. G.; Kim, H.; Suh, P.-G.; Choi, W. C. *Biochem. Soc. Trans.* **1991**, *19*, 337–341.

54. Rhee, S. G.; Ryu, S. H.; Lee, K. Y.; Cho, K. S. *Meth. Enzymol.* **1991**, *197*, 502–511.

55. Takenawa, T.; Nagai, Y. *J. Biol. Chem.* **1981**, *256*, 6769–6775.

56. Low, M. G.; Carroll, R. C.; Cox, A. C. *Biochem. J.* **1986**, *237*, 139–145.

57. Takenawa, T.; Homma, Y.; Emori, Y. *Meth. Enzymol.* **1991**, *197*, 511–518.

58. Bennett, C. F.; Angioli, M. P.; Crooke, S. T. *Meth. Enzymol.* **1991**, *197*, 526–535.

59. Fukui, T.; Lutz, R. J.; Lowenstein, J. M. *J. Biol. Chem.* **1988**, *263*, 17730–17737.

60. Banno, Y.; Yada, Y.; Nozawa, Y. *J. Biol. Chem.* **1988**, *263*, 11459–11465.

61. Herrero, C.; Cornet, M. E.; Lopez, C.; Barreno, P. G.; Municio, A. M.; Moscat, J. *Biochem. J.* **1988**, *255*, 807–812.

62. Manne, V.; Kung, H. F. *Biochem. J.* **1987**, *243*, 763–771.

63. Nozawa, Y.; Banno, Y. *Meth. Enzymol.* **1991**, *197*, 518–526.

64. Wilson, D. B.; Bross, T. E.; Sherman, W. R.; Berger, R. A.; Majerus, P. W. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4013–4017.

65. Wilson, D. B.; Connolly, T. M.; Bross, T. E.; Majerus, P. W.; Sherman, W. R.; Tyler, A. N., Rubin, L. J.; Brown, J. E. *J. Biol. Chem.* **1985**, 260, 13496–13501.

66. McDonald, L. J.; Mamrack, M. D. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 203–208

67. Homma, Y.; Emori, Y.; Shibasaki, F.; Suzuki, K.; Takenawa, T. *Biochem. J.* **1990**, *269*, 13–18.

68. Hoffmann, B.; Seib, C.; Hoer, A.; Hoer, D.; Oberdisse, E.; Rosenthal, W.; Schultz, G. *Biomed. Biochim. Acta* **1991**, *50*, 31–46.

69. Moriyama, T.; Narita, H.; Oki, M.; Matsuura, T.; Kito, M. *J. Biochem. (Tokyo)* **1990**, *108*, 414–419.

70. Morris, A. J.; Waldo, G. L.; Downes, C. P.; Harden, T. K. *J. Biol. Chem.* **1990**, *265*, 13501–13507.

71. Morris, A. J.; Waldo, G. L.; Downes, C. P.; Harden, T. K. *J. Biol. Chem.* **1990**, *265*, 13508–13514

72. Nakanishi, O.; Homma, Y.; Kawasaki, H.; Emori, Y.; Suzuki, K.; Takenawa, T. *Biochem. J.* **1988**, *256*, 453–459.

73. Perrella, F. W.; Jankewicz, R.; Dandrow, E. A. *Biochim. Biophys. Acta* **1991**, *1076*, 209–214.

74. Roy, G.; Villar, L. M.; Lazaro, I.; Gonzalez, M.; Bootello, A.; Gonzalez–Porque, P. *J. Biol. Chem.* **1991**, *266*, 11495–11501.

75. Shaw, K.; Exton, J. H. *Biochemistry* **1992**, *31*, 6347–6354.

76. Tompkins, T. A.; Moscarello, M. A. *J. Biol. Chem.* **1991**, *266*, 4228–4236.

77. Wightman, P. D.; Dahlgren, M. E.; Hall, J. C.; Davies, P.; Bonney, R. J. *Biochem. J.* **1981**, *197*, 523–526.

78. Wilson, D. B.; Bross, T. E.; Hofmann, S. L.; Majerus, P. W. *J. Biol. Chem.* **1984**, *259*, 11718–11724.

79. Kim, J. W.; Ryu, S. H.; Rhee, S. G. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 177–182.

80. Majerus, P. W.; Connolly, T. M.; Bansal, V. S.; Inhorn, R. C.; Ross, T. S.; Lips, D. L. *J. Biol. Chem.* **1988**, *263*, 3051–3054.

81. Cruz–Rivera, M.; Bennett, C. F.; Crooke, S. T. *Biochim. Biophys. Acta* **1990**, *1042*, 113–118.

82. Holub B. J.; Celi, B. *Can. J. Biochem. Cell. Biol.* **1984**, *62*, 115–120.

83. Ikezawa, H.; Yamanegi, M.; Taguchi, R.; Miyashita, T.; Ohyabu, T. *Biochim. Biophys. Acta* **1976**, *450*, 154–164.

84. Taguchi, R.; Ikezawa, H. *Arch. Biochem. Biophys.* **1978**, *186*, 196–201.

85. Sundler, R.; Alberts, A. W.; Vagelos, P. R. *J. Biol. Chem.* **1978**, *253*, 4175–4179.

86. Ikezawa, H.; Nakabayashi, T.; Suzuki, K.; Nakajima, M.; Taguchi, T.; Taguchi, R. *J. Biochem. (Tokyo)* **1983**, *93*, 1717–1719.

87. Ikezawa, H.; Taguchi, R. *Meth. Enzymol.* **1981**, *71*, 731–741.

88. Griffith, O. H.; Volwerk, J. J.; Kuppe, A. *Meth. Enzymol.* **1991**, *197*, 493–502.

89. Low, M. G. *Meth. Enzymol.* **1981**. *71*, 741–746.

90. Goldfine, H.; Knob, C. *Infect. Immun.* **1992**. *60*, 4059–4067.

91. Volwerk, J. J.; Koke, J. A.; Wetherwax, P. B.; Griffith, O. H. *FEMS Microbiol. Lett.* **1989**, *61*, 237–241.

92. Jaeger, K.; Stieger, S.; Brodbeck, U. *Biochim. Biophys. Acta* **1991**, *1074*, 45–51.

93. Volwerk, J. J.; Shashidhar, M. S., Kuppe; A.; Griffith, O. H. *Biochemistry* **1990**, *29*, 8056–8062.

94. Bruzik, K. S.; Morocho, A. M.; Jhon, D. Y.; Rhee, S. G.; Tsai, M.-D. *Biochemistry* **1992**, *31*, 5183–5193.

95. Ferguson, M. A. J.; Homans, S. W.; Dwek, R. A.; Rademacher, T. W. *Science* **1987**, *239*, 753–759.

96. Futerman, A. H.; Low, M. G.; Ackerman, K. E.; Sherman, W. R.; Silman, 1. *Biochem. Biophys. Res. Commun.* **1985**, *129*, 312–317.

97. Larner, J.; Huang, L. C.; Schwartz, C. F. W.; Oswald, A. S.; Shen, T.-Y.; Kinter, M.; Tang, G.; Zeller, K. *Biochem. Biophys. Res. Commun.* **1988**, *151*, 1416–1426.

98. Larner, J.; Huang, L. C.; Tang, G.; Suzuki, S.; Schwartz, C. F. W.; Romero, G.; Roulidis, Z.; Zeller, K.; Shem, T. Y.; Oswald, A. S.; Luttrell, L. *Cold Spring Harbor Symp. Quant. Biol.* **1988**, 965–971.

99. Roberts, W. L.; Myher, J. J.; Kuksis, A.; Low, M. G.; Rosenberry, T. L. *J. Biol. Chem.* **1988**, *263*, 18766–18775.

100. Ferguson, M. A. J. *Biochem. J.* **1992**, *284*, 297–300.

101. Field, M. C. *Glycoconjugate J.* **1992**, *9*, 155–159.

102. Stieger, S.; Brodbeck, U. *Biochimie* **1991**, *73*, 1179–1186.

103. Hereld, D.; Hart, G. W.; Englund, P. T. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8914–8918.

104. Carrington, M.; Buelow, R.; Reinke, H.; Overath, P. *Mol. Biochem. Parasitol.* **1989**, *33*, 289–296.

105. Mensa-Wilmot, K.; Englund, P. T. *Mol. Biochem. Parasitol.* **1992**, *56*, 311–321.

106. Fox, J. A.; Duszenko, M.; Ferguson, M. A. J.; Low, M. G.; Cross, G. A. M. *J. Biol. Chem.* **1986**, *261*, 15767–15771.

107. Fox, J. A.; Soliz, N. M.; Saltiel, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2663–2667.

108. Fouchier, F. Baltz, T.; Rougon, G. *Biochem. J.* **1990**, *269*, 321–327.

109. Stieger, S.; Diem, S.; Jakob, A.; Brodbeck, U. *Eur. J. Biochem.* **1991**, *197*, 67–73.

110. Buelow, R.; Overath, P. *J. Biol. Chem.* **1986**, *261*, 11918–11923.

111. Cubitt, A. B.; Firtel, R. A. *Biochem. J.* **1992**, *283*, 371–378.

112. Toyoshima, S.; Matsumoto, N.; Wang, P.; Inoue, H.; Yoshioka, T.; Hotta, Y.; Osawa, T. *J. Biol. Chem.* **1990**, *265*, 14842–14848.

113. Melin, P. M.; Pical, C.; Jergil, B.; Sommarin, M. *Biochim. Biophys. Acta* **1992**, *1123*, 163–169.

114. Tate, B. F.; Schaller, G. E.; Sussman, M. R.; Crain, R. C. *Plant Physiol.* **1989**, *91*, 1275–1279.

115. (a) McMurray, W. C.; Irvine, R. F. *Biochem. J.* **1988**, *249*, 877–881. (b) Buetikofer, P.; Brodbeck, U. *J. Biol. Chem.* **1993**, *268*, 17794–17802.

116. Koke, J. A.; Yang, M.; Henner, D. J.; Volwerk, J. J.; Griffith, O. H. *Prot. Expression Purif.* **1991**, 2, 51–58.

117. Lapetina, G. E.; Michell. R. H. *Biochem. J.* **1973**, *131*, 433–442.

118. Ross, T. S.; Majerus, P. W. *J. Biol. Chem.* **1992**, *267*, 19924–19928.

119. Ross, T. S.; Wang, F. P.; Majerus, P. W. *J. Biol. Chem.* **1992**, *267*, 19919–19923.

120. Goldfine, H.; Johnston, N. C.; Knob, C. *J. Bacteriol.* **1993**, *175*, 4298–4306.

121. Dawson, R. M. C.; Freinkel, N.; Jungalwala, F. B.; Clarke, N. *Biochem. J.* **1971**, *122*, 605–607.

122. Quinn, P. J. In *Cyclitols; Phosphoinositides* pp. 399–419, Wells, W. W.; Eisenberg, F. Eds., Academic Press, New York, 1978.

123. Lin, G.; Bennett, C. F.; Tsai; M.-D. *Biochemistry* **1990**, *29*, 2747–2757.

124. Lin, G. Tsai, M.-D. *J. Am. Chem. Soc.* **1989**, *111*, 3099–3101.

125. Bruzik, K. S.; Lin, G.; Tsai, M.-D. In *Inositol Phosphates and Derivatives. Synthesis, Biochemistry and Therapeutic Potential* pp. 172–185, *ACS Symp. Ser.* **463**, Reitz, A. B. Ed., 1991.

126. Bruzik, K. S.; Tsai, M.-D. *Meth. Enzymol.* **1991**, *197*, 258–269.

127. Knowles, J. R. *Ann. Rev. Biochem.* **1980**, *49*, 877.

128. Eckstein, F.; Romaniuk, P. J.; Connolly, B. A. *Meth. Enzymol.* **1982**, *87*, 197–212.

129. Cleland, W. W. *Biochemistry* **1990**, *29*, 3194–3197.

130. Yang, S. F.; Freer, S.; Benson, A. A. *J. Biol. Chem.* **1967**, *242*, 477–484.

131. (a) Kanfer, J. N. *Can. J. Biochem.* **1980**, *58*, 1370–1380. (b) Wang, P.; Schuster, M.; Wang, Y.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 10487-10491.

132. Bruzik, K. S.; Tsai, M.-D. to be published, 1994.

133. Jain, M. K.; Gelb, M. H. *Meth. Enzymol.* **1991**, *197*, 112–125.

134. Hofmann, S. L.; Majerus, P. W. *J. Biol. Chem.* **1982**, *257*, 14359–14364.

135. Irvine, R. F.; Hemington, N.; Dawson, R. M. C. *Eur. J. Biochem.* **1979**, *99*, 525–530.

136. Jackowski, S.; Rock, C. O. *Arch. Biochem. Biophys.* **1989**, *268*, 516–524.

137. Jones, G. A.; Carpenter, G. *J. Biol. Chem.* **1993**, *268*, 20845–20850.

138. Rebecchi, M.; Eberhardt, R.; Delaney, T.; Ali, S.; Bittman, R. *J. Biol. Chem.* **1993**, *268*, 1735–1741.

139. Rebecchi, M.; Peterson, A.; McLaughlin, S. *Biochemistry* **1992**, *31*, 12742–12747.

140. Rebecchi, M.; Boguslavsky, V.; Boguslavsky, L.; McLaughlin, S. *Biochemistry* **1992**, *31*, 12748–12753.

141. Hirasawa, K.; Irvine, R. F.; Dawson, R. M. C. *Biochem. J.* **1981**, *193*, 607–614.

142. Chung, S. M.; Proia, A. D.;Klintworth, G. K.; Watson, S. P.; Lapetina, E. G. *Biochem. Biophys. Res. Commun.* **1985**, *129*, 411–416.

143. Kume, T.; Taguchi, R.; Ikezawa, H. *Chem. Pharm. Bull.* **1991**, *39*, 2063–2067.

144. Kume, T.; Taguchi, R.; Ikezawa, H. *Chem. Pharm. Bull.* **1991**, *39*, 2980–2983.

145. Rosenberry, T. L. to be published.

146. Panfoli, I.; Morelli, A.; Pepe, I. M. *Ital. J. Biochem.* **1992**, *41*, 16–25.

147. Artursson, E.; Puu, G. *Can. J. Microbiol.* **1992**, *38*, 1334–1337.

148. Creutz, C. E.; Dowling, L. G.; Kyger, E. M.; Franson, R. C. *J. Biol. Chem.* **1985**, *260*, 7171–7173.

149. McDonald, L. J.; Mamrack, M. D. *Biochemistry* **1989**, *28*, 9926–9932.

150. Banno, Y.; Suzuki, T.; Nozawa, Y. *Blood Rev.* **1992**, *2*, 69–77.

151. Griendling, K. K.; Taubman, M. B.; Akers, M.; Mendlovitz, M.; Alexander, R. W. *J. Biol. Chem.* **1991**, *266*, 15498–15504.

152. Chien, M. M.; Cambier, J. C. *J. Biol. Chem.* **1990**, *265*, 9201–9207.

153. Ikezawa, H.; Yamanegi, M.; Taguchi, R.; Miyashita, T.; Ohyabu, T. *Biochim. Biophys. Acta* **1976**, *450*, 154–164.

154. Thatcher, G. R. J.; Campbell, A. S. *J. Org. Chem.* **1993**, *58*, 2272–2281.

155. Reynolds, L. J.; Washburn, W. N.; Deems, R. A.; Dennis, E. A. *Meth. Enzymol.* **1991**, *197*, 3–31.

156. Rittenhouse, S. E. *Meth. Enzymol.* **1982**, *86*, 3–11.

157. Hanley, M. R.; Poyner, D. R.; Hawkins, P. T. *Meth. Enzymol.* **1991**, *197*, 149–158.

158. Shashidhar, M. S.; Volwerk, J. J.; Griffith, O. H.; Keana, J. F. W. *Chem. Phys. Lipids* **1991**, *60*, 101–110.

159. Leigh, A. J.; Volverk, J. J.; Griffith, O. H.; Keana, J. F. W. *Biochemistry* **1992**, *31*, 8978–8983.

160. Hendrickson, E. K.; Johnson, J. L.; Hendrickson, H. S. *BioMed. Chem. Lett.* **1991**, *1*, 615–618.

161. Hendrickson, E. K.; Hendrickson, H. S.; Johnson, J. L.; Khan, T. H.; Chial, H. J. *Biochemistry* **1992**, *31*, 12169–12172.

162. Brocklehurst, K. *Meth. Enzymol.* **1982**, *87*, 427–469.

163. Cox, J. W.; Snyder, W. R.; Horrocks, L. A. *Chem. Phys. Lipids* **1979**, *25*, 369–380.

164. Young, P. R.; Snyder, W. R.; McMahon, R. F. *Biochem. J.* **1991**, *280*, 407–410.

165. Yu, L.; Dennis, A. A. *Methods Enzymol.* **1991**, *197*, 65–75.

166. (a) Alisi, M. A.; Brufani, M.; Filocamo, L.; Gostoli, G.; Lappa, S. Maiorana, S.; Cesta, M. C.; Ferrari, E.; Pagella, P. G. *Tetrahedron Lett.* **1992**, *33*, 3891–3894. (b) Hendrickson, H. S. to be published.

167. Rawyler, A.; Siegenthaler, P. A. *Biochim. Biophys. Acta* **1989**, 1004, 337–344.

168. Maslanski, J. A.; Busa, W. B. In *Methods in Inositide Research* pp. 113–126, Irvine, R. F., Ed., Raven Press, New York, 1990.

169. Hendrickson, E. K.; Johnson, J. L.; Hendrickson, H. S. *BioMed. Chem. Lett.* **1991**, *1*, 619–622.

170. Gadella, Jr, T. W. J.; Moritz, A.; Westerman, J.; Wirtz, K. W. A. *Biochemistry* **1990**, *29*, 3389–3395.

171. Kaplun, A. P.; Shragin, A. S.; Lyutik, A. I.; Shvets, V. I.; Evstigneeva, R. P. *Dokl. Akad. Nauk SSSR* **1983**, *273*, 350–351.

172. Shashidhar, M. S.; Volwerk, J. J.; Keana, J. F. W.; Griffith, O. H. *Anal. Biochem.* **1991**, *198*, 10–14.

173. Bangham, A. D.; Dawson, R. M. C. *Biochem. J.* **1960**, *75*, 133–138.

174. Ransac, S.; Moreau, H.; Riviere, C.; Verger. R. *Meth. Enzymol.* **1991**, *197*, 49–65.

175. Dennis E. A.; Plueckthun, A. In *Phosphorus-31 NMR*, pp. 423–446, Gorenstein, D. G., Ed., Academic Press, Orlando, 1984.

176. Roberts, M. F. *Meth. Enzymol.* **1991**, *197*, 31–48.

177. Leavitt, A. L.; Sherman, W. R. *Meth. Enzymol.* **1982**, *89*, 9–18.

178. Sun, G. Y.; Lin, T.-N. In *Methods in Inositide Research* pp. 153–158, Irvine, R. F. Ed., Raven Press, New York, 1990.

179. Irvine, R. F. *Methods in Inositide Research*, Raven Press, New York, 1990.

180. Mayr, G. W. *Biochem. J.* **1988**, *254*, 585–591.

181. Mayr, G. W. In *Methods in Inositide Research* pp. 83–108, Irvine, R. F. Ed., Raven Press, New York, 1990.

182. Sun, G. Y.; Lin, T.-N; Premkumar, N.; Carter, S.; MacQuarrie, R. A. In *Methods in Inositide Research* pp. 135–143, Irvine, R. F. Ed., Raven Press, New York, 1990.

183. Young, R. C.; Downes, C. P. *Drug Design Del.* **1990**, *6*, 1–13.

184. Billington, D. C. *Chem. Soc. Rev.* **1989**, *18*, 83–122.

185. Billington, D. C. In *The Inositol Phosphates. Chemical Synthesis and Biological Significance* pp. 95–138, VCH, Weinheim, 1993.

186. Potter, B. L. V. *Nat. Prod. Rep.* **1990**, 1–24.

187. Young, R. C.; Downes, C. P.; Eggleston, D. S.; Jones, M.; Macphee, C. H.; Rana, K. K.; Ward; J. G. *J. Med. Chem.* **1990**, *33*, 641–646

188. Ward, J. G.; Young, R. C. In *Inositol Phosphates and Derivatives. Synthesis, Biochemistry and Therapeutic Potential, ACS Symp. Ser. 463*, 214–228.

189. Kozikowski, A. P.; Ognyanov, V. I.; Fauq, A. H.; Nahorski, S. R.; Wilcox, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 4429–4434.

190. Kume, T.; Taguchi, R.; Tomita, M.; Tokuyama, S.; Morizawa, K.; Nakachi, O.; Hirano, J.; Ikezawa, H. *Chem. Pharm. Bull.* **1992**, *40*, 2133–2137.

191. Kaufmann, F.; Massy, D. J. R.; Pirson, W.; Wyss. P. In *Inositol Phosphates and Derivatives. Synthesis, Biochemistry and Therapeutic Potential, ACS Symp. Ser. 463*, 202–213.

192. Shashidhar, M. S.; Keana, J. F. W.; Volwerk, J. J.; Griffith, O. H. *Chem. Phys. Lipids* **1990**, *53*, 103–113.

193. Shashidhar, M. S.; Volwerk, J. J.; Keana, J. F. W.; Griffith, O. H. *Biochim. Biophys. Acta* **1990**, *1042*, 410–412.

194. Cifuentes, M.; Delaney, T; Rebecchi, M. J. *Abstracts of Am. Soc. Biochem. Mol. Biol.* May 30-June 3, 1993, San Francisco.

195. Mato, J. M.; Kelly, K. L.; Abler, A.; Jarett, L.; Corkey, B. E.; Cashel, J. A.; Zopf, D. *Biochim. Biophys. Res. Commun.* **1987**, *146*, 764–770.

196. Bruzik, K. S.; Tsai, M.-D. to be published, 1994.

197. Massy, D. J. R.; Wyss, P. *Helv. Chim. Acta* **1990**, *73*, 1037–1057.

198. Seitz, S. P.; Kaltenbach lll, R. F.; Vreekamp, R. H.; Calabrese, J. C.; Perrella, F. W. *BioMed. Chem. Lett.* **1992**, *2*, 171–174.

199. Schedler, D. J. A. PhD Dissertation, University of Alabama, *Dissertation Abstr. Intl.* **1992**, *53B*, 4118.

200. Schedler, D. J. A.; Rhee, S. G.; Baker, D. C. to be published.

201. Rosenberry, T. L. *Cell Biol. Int. Rep.* **1991**, *15*, 1133–1149.

202. Lips, D. L.; Majerus, P. W.; Gorga, F. R.; Young, A. T.; Benjamin, T. L. *J. Biol. Chem.* **1989**, *264*, 8759–8763.

203. Serunian, L. A.; Haber, M. T.; Fukui, T.; Kim, J. W.; Rhee, S. G.; Lowenstein, J. M.; Cantley, L. C. *J. Biol. Chem.* **1989**, *264*, 17809–17815.

204. Brufani, M.; Cesta, M. C.; Donnarumma, L.; Filocamo, L.; Gostoli, G.; Lappa, S.; Ferrari, E.; Pagella, S. *Carbohydr. Res.* **1992**, *228*, 371–376.

205. Marta, M.; Orlando, P.; Pomponi, M.; Pagella, P. G. *Acta Med. Rom.* **1988**, *26*, 429–432.

206. Dreef, C. E.; Douwes, M.; Elie, C. J. J.; van der Marel, G. A.; van Boom, J. H. *Synthesis* **1991**, 443–447.

207. Dreef, C. E.; Elie, C. J. J.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1991**, *32*, 955–958.

208. Elie, C. J. J.; Brounts, D. M.; Dreef, C. E.; van der Marel, G. A.; van Boom, J. H. *Rec. Trav. Chim. Pays-Bas* **1992**, *111*, 192–196.

209. Elie, C. J. J.; Brounts, D. M.; Dreef, C. E.; van der Marel, G. A.; van Boom, J. H. *Rec. Trav. Chim. Pays-Bas* **1991**, *110*, 192–196.

210. Campbell, S. A.; Thatcher, G. R. J. *Tetrahedron Lett.* **1991**, *32*, 2207–2210.

211. Cesta, M. C.; Filocamo, L.; Lappa, S. *Synth. Commun.* **1991**, 21, 1551–1554.

212. Lipsky, J. J.; Lietman, P. S. *J. Pharm. Exp. Ther.* **1982**, *220*, 287–292.

213. Downes, C. P.; Michell, R. H. *Biochem. J.* **1981**, *198*, 133–140.

214. Nahas, N.; Graff, G. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1035–1040.

215. Sagawa, N.; Bleasdale, J. E.; Di Renzo, G. C. *Biochim. Biophys. Acta* **1983**, *752*, 153–161.

216. Ji, Y.-H.; Pantze, M.; Pirson, W.; Wyss, P. paper presented at Xllth International Symposium on Medicinal Chemistry, Basel, Sept. 13–17, 1992.

217. Powis, G.; Seewald, M. J.; Gratas, C.; Melder, D.; Riebow, J.; Modest, E. J. *Cancer Res.* **1992**, *52*, 2835–2840.

218. Aoki, M.; Itezono, Y.; Shihari, H.; Nakayama, N.; Sakai, A.; Tanaka, Y.; Yamaguchi, A.; Shimma, N.; Yokose, K.; Seto, H. *Tetrahedron Lett.* **1991**, *32*, 4737–4740.

219. Bennett, C. F.; Mong, S.; Wu, H.-L. W.; Clark, M. A.; Wheeler, L.; Crooke, S. T. *Mol. Pharm.* **1987**, 32, 587–593.

220. Kyger, E. M.; Franson, R. C. *Biochim. Biophys. Acta* **1984**, *794*, 96–103.

221. Shvets, V. I. *Russ. Chem. Rev.* **1974**, *43*, 488–502.

222. Stepanov, A. E.; Shvets, V. l. *Chem. Phys. Lipids* **1979**, *25*, 247–263.

223. Gigg, R. *Chem. Phys. Lipids* **1980**, 26, 287.

224. Ward, J. G.; Young, R. *Tetrahedron Lett.* **1988**, *29*, 6013–6016.

225. Salamonczyk, G. M.; Bruzik, K. S. *Tetrahedron Lett.* **1990**, 2015–2016.

226. Jones, M.; Rana, K. K.; Ward, J. G.; Young, R. C. *Tetrahedron Lett.* **1989**, *30*, 5353–5356.

227. Ozaki, S.; Watanabe, Y.; Ogasawara, T.; Kondo, Y.; Shiotani, N.; Nishii, H.; Matsuki, T. *Tetrahedron Lett.* **1986**, *27*, 3157.

228. Dreef, C. E.; Elie, C. J. J.; Hoogerhout, P.; van der Marel, G. A.; van Boom, J. H., *Tetrahedron Lett.* **1988**, *29*, 6513–6516.

229. Somerharju, P.; Wirtz, K. W. A. *Chem. Phys. Lipids* **1982**, *30*, 81.

230. Noda, N.; Keenan, R. W. *Chem. Phys. Lipids* **1990**, *53*, 53–63.

231. Garigapati, Z. R.; Roberts, M. F. *Tetrahedron Lett.* **1993**, *34*, 769–772.

232. Bullock, T. L.; Ryan, M.; Kim, S.; Remington, S. J.; Griffith, O. H. *Biophys. J.* **1993**, *64*, 784–791.

233. Jhon, D.-Y.; Lee, H.-H.; Park, D.; Lee, C.-W.; Lee, K.-H.; Yoo, O. J.; Rhee, S. G. *J. Biol. Chem.* **1993**, *268*, 6654–6661.

234. Hofmann, S. L.; Majerus, P. W. *J. Biol. Chem.* **1982**, *257*, 6461–6469.

235. Bennett, C. F.; Balcarek, J. M.; Varrichio, A.; Crooke; S. T. *Nature* **1988**, *334*, 268–270.

236. Srivastava, S. P.; Chen, N.; Liu, Y.; Holtzman, J. L. *J. Biol. Chem.* **1991**, *266*, 20337–20344.

237. Ryu, S. H.; Cho, K. S.; Lee, K.-Y.; Suh, P.-G.; Rhee, S. G. *Biochim. Biophys. Res. Commun.* **1986**, *141*, 137–144.

238. Katan, M.; Parker, P. J. *Eur. J. Biochem.* **1987**, *168*, 413–418.

239. Suh, P.-G.; Ryu, S. H.; Moon, K. H.; Suh, H. W.; Rhee, S. G. *Cell* **1988**, *54*, 161–169.

240. Ohta, S.; Matsui, A.; Nazawa, Y.; Kagawa, Y. *FEBS Lett.* **1988**, *242*, 31–35.

241. Emori, Y.; Homma, Y.; Sorimachi, H.; Kawasaki, H.; Nakanishi, O.; Suzuki, K.; Takenawa, T. *J. Biol. Chem.* **1989**, *264*, 21885–21890.

242. Stahl, M. L.; Ferenz, C. R.; Kelleher, K. L.; Kriz, R. W.; Knopf, J. L. *Nature* **1988**, *332*, 269–272.

243. Meldrum, E.; Katan, M.; Parker, P. *Eur. J. Biochem.* **1989**, *182*, 673–677.

244. Meldrum, E.; Kriz, R. W.; Totty, N.; Parker, P. J. *Eur. J. Biochem.* **1991**, *196*, 159–165.

245. Thomas, G. M. H.; Geny, B.; Cockroft, S. *EMBO J.* **1991**, *10*, 2507–2512.

246. Leimeister-Waechter, M.; Domann, E.; Chakraborty, T. *Mol. Microbiol.* **1991**, *5*, 361–366.

247. Kuppe, A.; Hedberg, K. K.; Volwerk, J. J.; Griffith, O. H. *Biochim. Biophys. Acta* **1990**, *1047*, 41–48.

248. Cifuentes, M.; Honkanen, L.; Rebecchi, M. J. *J. Biol. Chem.* **1993**, *268*, 11586–11593.

249. Park, D.; Jhon, D.-Y.; Lee, C.-W.; Ryu, S. H.; Rhee, S. G. *J. Biol. Chem.* **1993**, *268*, 3710–3714.

250. Panfoli, I.; Morelli, A.; Pepe, I. M. *Ital. J. Biochem.* **1992**, *41*, 147–158.

251. Banno, Y.; Nakashima, S.; Nozawa, Y. *Biochim. Biophys. Res. Commun.* **1986**, *136*, 713–721.

252. Banno, Y.; Yu.; Nakashima, T.; Homma, Y.; Takenawa, T.; Nozawa Y. *Biochim. Biophys. Res. Commun.* **1990**, *167*, 396–401.

253. Kozawa, O.; Hoshijima, M.; Tanimoto, T.; Ohmori, T.; Takai, Y. *Biochim. Biophys. Res. Commun.* **1987**, *145*, 218–227.

254. Kupke, T.; Lechner, M.; Kaim, G.; Goetz, F. *Eur. J. Biochem.* **1989**, *185*, 151–155.

255. Lechner, M.; Kupke, T.; Stefanovic, S.; Gotz, F. *Mol. Microbiol.* **1989**, *3*, 621–626.

256. Mengaud, J.; Breton–Braun, C.; Cossart, P. *Mol. Microbiol.* **1991**, *5*, 367–372.

257. Low, M. G.; Finean, J. B. *Biochem. J.* **1976**, *154*, 203–208.

258. Low, M. G.; Finean, J. B. *Biochem. J.* **1977**, *162*, 235–240.

259. Henner, D. J.; Yang, M.; Chen, E.; Hellmiss, R.; Rodriguez, H.; Low, M. G. *Nucl. Acids Res.* **1988**, *16*, 10383.

260. Bloomquist, B. T.; Shortridge, R. D.; Schneuwly, S.; Perdew, M.; Montell, C.; Steller, H.; Rubin, G.; Pak, W. L. *Cell* **1988**, *54*, 723–733.

261. Shortridge, R. D.; Yoon, J.; Lending, C. R.; Bloomquist, B. T.; Perdew, M. H.; Pak, W. L. *J. Biol. Chem.* **1991**, *266*, 12474–12480.

262. Suh, P.-G.; Ryu, S. H.; Moon, K. H.; Suh, H. W.; Rhee, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5419–5423.

263. Drayer, A. L.; van Haastert, P. J. M. *J. Biol. Chem.* **1992**, *267*, 18387–18392.

264. Lombardo, D.; Dennis, E. A. *J. Biol. Chem.* **1985**, *260*, 7234–7240.

265. Bruzik, K. S.; Tsai, M.-D. *J. Am. Chem. Soc.* **1992**, *114*, 6361–6374.

Biographical Sketch of Karol S. Bruzik

Karol S. Bruzik became Assistant Professor of Medicinal Chemistry at the University of Illinois at Chicago in 1993. He received a PhD degree working with Wojciech J. Stec at the Polish National Academy of Sciences in 1980. Between 1981 and 1983 he had a postdoctoral position in Ming–Daw Tsai's lab. He became Assistant Professor at the Center of Molecular and Macromolecular Studies in Lodz, Poland in 1988, but returned to The Ohio State University in 1990 as a Research Scientist. His research interests include the synthesis of analogs of biomolecules as probes and inhibitors of enzymes, the mechanism of enzyme reactions, NMR of biomolecules, and generalization of self-assembly of amphiphilic molecules.

Biographical Sketch of Ming-Daw Tsai

Ming-Daw Tsai was born in Taiwan, Republic of China in 1950, and received a BS in chemistry from the National Taiwan University in 1972. After two years of military service, he went to Purdue University; received a PhD working with Heinz G. Floss in 1978. He joined the Chemistry Department at The Ohio State University in 1981 where he is now Professor of Chemistry and Biochemistry. He has also spent research leaves in the laboratories of Eric Oldfield, Sture Forsen and John Markley. His research interest lies in the interface between chemistry and biology, particularly in phosphorus stereochemistry, phospholipid biochemistry, structure–function relationships of phospholipases and kinases, and the use of NMR in biological systems.

(*Received* 13 *October* 1993; *accepted* 23 *November* 1993)