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Studies of Inositol Phospholipid–Specific Phospholipase C

SUE GOO RHEE, PANN-GHILL SUH, SUNG-HO RYU, SANG YEOL LEE

Inositol phospholipid–specific phospholipase C is the enzyme that generates phosphoinositide-derived messenger molecules. Mammalian cells contain at least five immunologically distinct phospholipase C enzymes that appear to be separate gene products. Complete amino acid sequences of four of these isozymes have been established. The overall sequence similarity is surprisingly low for enzymes catalyzing the same chemical reaction: three of them show limited amino acid sequence similarity to each other in two narrow regions, and the fourth enzyme is completely different. The diversity in primary structure together with different regional and cellular expression of the isozymes suggests that each isozyme has a defined function in processing the physiological response of different cell types to a variety of external stimuli and that each is regulated differently.

MUCH PROGRESS HAS BEEN MADE RECENTLY IN UNDERstanding the process by which cells respond to extracellular signals. These extracellular signals are transmitted across the cell membrane by a variety of mechanisms that use second messenger molecules. Hormones, growth factors, neurotransmitters, and other agonists bind to specific receptors on the external surface of a cell. Receptor occupancy initiates the production of active second messengers, including the well-characterized adenosine 3′,5′-monophosphate (cAMP) and guanosine 3′,5′-monophosphate molecules, as well as the more recently identified messenger molecules, diacylglycerol and inositol phosphates, that are derived from inositol phospholipids (*1–3*). Second messengers, once formed, evoke a host of intracellular reactions that eventually lead to many cellular processes such as metabolism, excitation, secretion, contraction, sensory mechanism, and cell growth.

Phospholipase C Isozymes

The phospholipase C (PLC) isozymes, which cleave inositol phospholipids to diacylglycerol and inositol phosphates, are present in most mammalian cells as well as in plants and various microorganisms (*4*). The first PLC purified to homogeneity was from rat liver; when subjected to SDS–polyacrylamide gel electrophoresis (PAGE), its molecular mass was 68 kD (*5*). Subsequently, numerous PLC activities have been resolved chromatographically from a variety of tissues and shown to differ in molecular mass, isoelectric point and *p*H optima, and calcium dependency, indicating the existence of PLC isozymes (*6*). Definitive proof of the presence of PLC isozymes was derived from the two distinct enzymes purified from sheep seminal vesicles (*7*). One enzyme purified to homogeneity was 65 kD on SDS-PAGE; the other enzyme, which was partially purified, was 85 kD when estimated by gel filtration techniques. Antibodies to each of these enzymes did not react with the other.

Three PLC isozymes of 150, 145, and 85 kD were recently purified to homogeneity from bovine brain (*8, 9*). Both polyclonal and a series of monoclonal antibodies to the three enzymes were prepared and characterized (*9, 10*). The characterization included the evaluation of immunoreactivity toward each of the PLC forms in their native and denatured states. Each antibody reacted only with the enzyme against which it was prepared.

The three enzymes were specific for phosphatidylinositol (PI) and the polyphosphoinositides (*9*) and did not hydrolyze other phospholipids. We studied the catalytic properties of the three isozymes by using small unilamellar vesicles prepared from either PI or phosphatidylinositol-4,5-bisphosphate (PIP_2) as substrates. Hydrolysis of both PI and PIP_2 by the three enzymes was dependent on Ca^{2+}. However, at low Ca^{2+} concentration, PIP_2 was the preferred substrate for all three enzymes. When PI was the substrate, the three enzymes had similar specific activities at their optimum *p*H, which was 4.8 for the 150-kD form, 5.0 for 145-kD form, and 5.5 for 85-kD form. But at neutral *p*H, the order of specific activity was 85-kD PLC > 145-kD PLC > 150-kD PLC. In contrast, the order of specific activity for PIP_2 hydrolysis was 150-kD PLC > 85-kD PLC > 145-kD PLC, meaning that the 150-kD enzyme is the most specific for PIP_2.

Several more PLC enzymes have been purified to homogeneity (Table 1). These include a 62-kD form from guinea pig uterus (*11*),

S. G. Rhee is at the Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892. Present address for P.-G. Suh and S.-H. Ryu is Department of Life Science, Pohang Institute of Science and Technology, Pohang, Korea. Present address for S. Y. Lee is Department of Biochemistry, Kyongsang National University, Chinju, Korea.

a 154-kD form from bovine brain (*12*), an 88-kD form from bovine brain (*13*), two 85-kD forms from rat brain (*14*), a 61-kD form from human platelets (*15*), and an 87-kD form from rat liver (*16*). The two 85-kD enzymes purified from bovine brain are not only distinct immunochemically but also catalytically. One form, designated as PLC-III by Homma *et al.* (*14*), was significantly more specific to PIP_2 than the other form (named PLC-II by Homma *et al.*) in the presence of micromolar concentrations of Ca^{2+}. Initially, we named the 150-, 145-, and 85-kD enzymes from bovine brain PLC-I, PLC-II, and PLC-III, respectively. The PLC-II described by Homma *et al.* (*14*) appears to be identical to the 85-kD PLC-III we previously identified on the basis of catalytic properties. The 88-kD enzyme by Rebecchi and Rosen (*13*) was recognized by our antibody against the 85-kD enzyme and also had the same molecular mass on SDS-PAGE. Rat brain PLC enzymes of 250 to 300 kD were identified earlier by gel filtration chromatography (*6*). These larger forms can be attributed to the dimerization of the 150- and 145-kD forms; under a nondenaturing condition, most of the 150-kD form and part of the 145-kD form exist as dimer (*8*).

Table 1. Isozymes of PLC purified from different tissues.

Source	Molecular mass		Nomenclature		Reference
	SDS-PAGE (kD)	cDNA (kD)	Original report	Proposed	
Rat liver	68		I	α	(*5*)
Sheep seminal vesicular gland	65		I	α	(*7*)
	~85		II	δ or ε	(*7*)
Bovine brain	150	138.2*	I	β-1	(*8, 9*)
	140			β-2	(*9*)
	100			β-3	(*8, 9*)
	145	148.4*	II	γ	(*8, 9*)
	85	85.8*	III	δ	(*9*)
Guinea pig uterus	62	56.6†	II	α	(*11*)
Bovine brain	154	138.6	PLC-154	β-1	(*12*)
	88			δ	(*13*)
Rat brain	85		II	δ	(*14*)
	85		III	ε	(*14*)
Human platelets	61		mPLC-II		(*15*)
Rat liver	87				(*16*)

*Calculated molecular mass based on amino acid sequence deduced from rat brain cDNA. †Calculated molecular mass based on amino acid sequence deduced from rat basophilic leukemic cell cDNA.

Nomenclature of PLC Isozymes

With so many unsystematically named PLC enzymes, the nomenclature of PLC isozymes in the literature is in disarray. To clarify the relations of the enzymes listed in Table 1, we carried out a series of immunoblotting experiments and used three antibodies to bovine brain enzymes (150-, 145-, and 85-kD forms) and another antibody to the 62-kD guinea pig uterus enzymes (*17*). Not one of the three purified bovine brain enzymes was recognized by the antibody to the 62-kD uterus enzyme. However, in the immunoblots obtained with partially purified rat liver and seminal vesicular enzymes, the antibody to the 62-kD PLC recognized a band with a molecular mass nearly identical to that of the uterus enzyme (*17*). These results suggest (i) that although the reported molecular mass of each of the PLC enzymes from liver, seminal vesicle, and uterus differs slightly, these enzymes might still have similar amino acid sequences within the same animal species, and (ii) that the 62- to 68-kD enzyme from liver, seminal vesicle, and uterus is immunologically distinct from the three enzymes from brain. None of the antibodies to the three brain enzymes recognized the ~62-kD protein band in partially purified PLC preparations from liver, seminal vesicle, and uterus separated by SDS-PAGE.

Some investigators designated PLC enzymes by specifying molecular masses measured by SDS-PAGE. For example, the 154-kD enzyme was designated PLC-154 (*12*). However, uncertainty in the estimation of molecular mass and possible proteolytic cleavage make this method of nomenclature untenable. For example, recent nucleotide sequence data indicate that our 150-kD enzyme (*18*) and the enzyme named PLC-154 by Katan *et al.* (*19*) are identical, although the calculated molecular mass is on the order of 138.6 kD. Furthermore, antibodies to our 150-kD enzyme recognize two more proteins of 140 and 100 kD (*10, 20*). Both the purified 100- and 140-kD enzymes are as active catalytically as the 150-kD enzyme (*21*). Our experimental results suggest that the 100-kD enzyme is derived from the 150-kD form by the proteolytic action of calpain (*21*). However, it is not yet clear whether the 140-kD enzyme can be formed by proteolysis or by alternative splicing.

We have therefore started to use Greek letters to designate the PLC enzymes with different primary structures, assigning the letters according to the chronological order of their purification and using Arabic numerals after the Greek letters to designate PLC enzymes derived by proteolysis or alternative splicing (Table 1). The amino acid sequences of the three brain enzymes from rat are similar to those of bovine brain enzymes (*18, 19*). A large number of monoclonal antibodies to the three bovine brain enzymes recognize the corresponding enzymes from different mammalian species, tissues, and various cultured cells (*10*). It appears, therefore, that the differences in primary structure of a PLC from various mammalian species and from different tissues are not sufficient to hinder identification by this proposed nomenclature system.

There is not yet evidence to determine whether the 61-kD enzyme from human platelets is distinct from PLC-α. Likewise, the 87-kD enzyme from rat liver has not been differentiated from PLC-δ and PLC-ε.

Cytosolic PLC Compared with Membrane-Associated PLC

Numerous studies (*22*) suggest that the coupling of receptor function to PLC is mediated by an unspecified guanine nucleotide binding protein (G protein). This signal transduction model is analogous to the modulation of adenylate cyclase in which two G proteins, G_i and G_s, couple the receptor function to the synthesis of a second messenger molecule, cAMP (*23*). In addition, because adenylate cyclase and the modulating G proteins are membrane-bound proteins, the association of PLC with plasma membrane has been considered a prerequisite for the functional response of PLC to the signal transduced across the cell membrane. PLC activity has been demonstrated in various membrane preparations. Nevertheless, all PLC enzymes were initially purified from cytosolic fractions (*5, 7–11*).

The first membrane-associated PLC enzyme was purified from well-washed particulate fractions of bovine brain and shown to be the same as cytosolic PLC-β on the basis of the following observations (*20*): (i) The molecular masses were identical, (ii) all the monoclonal antibodies to the cytosolic PLC-β recognized the membrane-bound enzyme, and (iii) the elution profiles of tryptic peptides on a C_{18} reversed-phase column were similar, if not identical. The identical properties of the two enzymes were confirmed later by the fact that amino acid sequences deduced from the nucleotide sequences of rat cDNA for cytosolic PLC-β (*18*) and bovine cDNA for membrane-associated 154-kD enzyme (*19*) are

nearly identical. In other studies, immunoassay results indicated that significant quantities of PLC-α and PLC-γ are also associated with membrane fraction (*11, 24*). These findings suggest that specific mechanisms must be responsible for the translocation of PLC between cytosol and membrane. The translocation might constitute a way of regulating the PLC-dependent second messenger generation.

Primary Structures of PLC Isozymes

PLC-related cDNA clones corresponding to PLC-α, PLC-β, PLC-γ, and PLC-δ were isolated and sequenced. Each of these clones encodes a distinct polypeptide of 56.6 kD (PLC-α) (*25*), 138.2 kD (PLC-β) (*18, 19*), 148.4 kD (PLC-γ) (*26, 27*), and 85.8 kD (PLC-δ) (*18*). The cDNA clones corresponding to the PLC-β (*19*) and PLC-γ (*26*) were shown to encode functional enzymes when expressed in mammalian cells.

The four enzymes are quite dissimilar not only in molecular size but also in amino acid sequences. This lack of sequence similarity is consistent with the absence of immunological cross-reactivity between the four isozymes. When the sequences of enzymes β, γ, and δ (all of these sequences were deduced from nucleotide sequences of rat or bovine brain cDNAs) were compared, despite a low overall homology between the three enzymes, a significant sequence similarity was found in two regions, one of about 150 amino acids and the other of about 120 amino acids (*18*). The two domains, designated X and Y in Fig. 1, were 54% and 42% identical, respectively. Apparently, this magnitude of similarity is not enough to cause an immunological cross-reactivity. Regions X and Y are separated by 71, 407, and 50 amino acid residues in PLC-β, PLC-γ, and PLC-δ, respectively. From this, one might predict that the two X and Y regions constitute, separately or jointly, an important domain responsible for catalytic properties, such as the special recognition of phosphoinositides, the hydrolysis of phosphodiester bonds, or interaction with unidentified G proteins.

However, with the sequence of PLC-α (deduced from rat basophilic leukemic cell cDNA) becoming available, the prediction of the active site becomes more complex because the sequence of PLC-α shows no similarity to the sequences of the other three enzymes. This is perplexing because all four PLC isozymes have similar catalytic properties: they hydrolyze three known phosphoinositides, PI, PIP, and PIP_2 (PIP is phosphatidylinositol 4-monophosphate), but not phosphatidylcholine and phosphatidylethanolamine; and their catalytic activities are dependent on the concentration of Ca^{2+}. Nevertheless, a noticeable difference can be found in the Ca^{2+} dependence of the hydrolysis of polyphosphoinositides. The rates of hydrolysis of PIP and PIP_2 by PLC-α in the absence of Ca^{2+} but in the presence of EGTA were 25% to 33% of the rate observed for free calcium in a range of micromolar amounts (*11, 28*), whereas the rates of hydrolysis by the other three enzymes were nearly negligible in the absence of Ca^{2+} (*9, 12–14*). The PI hydrolysis was absolutely dependent on Ca^{2+} for all four enzymes. The 61-kD human platelet enzyme (*15*) and the 87-kD rat liver enzyme (*16*) (Table 1) are similar to PLC-α and PLC-δ, respectively, with respect to molecular mass and to the Ca^{2+} dependence of PIP_2 hydrolysis. Another significant difference between PLC-α and the other three PLCs (β, γ, and δ) is that the predicted amino acid sequence of PLC-α deduced from cDNA contained 24 amino acids at the NH_2-terminus, which showed a strong resemblance to the NH_2-terminal sequence and membrane-anchoring domain (*25*). The NH_2-terminal 24–amino acid sequence was absent in the cytosolic PLC-α, suggesting that proteolytic cleavage of the anchoring domain leads to translocation of PLC-α from membrane to cytosol. The amino acid sequences deduced for cDNAs corresponding to PLC-β, PLC-γ, and PLC-δ did not contain this anchoring domain at their termini (*18, 19, 26, 27*). However, significant amounts of PLC-β and PLC-γ were found associated with membrane, suggesting that PLC-β and PLC-γ may bind to proteins (for example, G protein) already attached to the lipid bilayer rather than being anchored directly to the membrane (*29*). Thus, it is tempting to speculate that there are two classes of phosphoinositide-specific PLC polypeptides, one class containing the conserved X and Y regions and another class without the two regions. This speculation will be substantiated if another PLC with a primary structure similar to PLC-α is discovered.

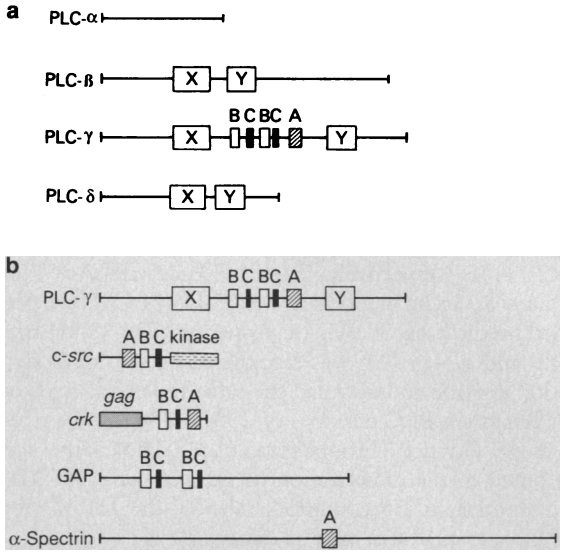

Fig. 1. (**a**) Linear representation of PLC isozymes. Open boxes X and Y each denote the regions of ~150 and ~120 amino acids, respectively, of similar sequence found in PLC-β (*18, 19*), PLC-γ (*26, 27*), and PLC-δ (*18*). The conserved regions are not found in PLC-α (*25*). (**b**) Structural similarities between PLC-γ, $pp60^{c\text{-}src}$ (*44*), $p47^{gag\text{-}crk}$ (*33*), GAP (*34*), and α-spectrin (*35*). The regions displaying sequence similarity are indicated by hatched box A, open box B, and black box C, which represent ~50, ~40, and ~15 amino acid residues, respectively.

Implication of Multiple PLC Forms

The mechanisms by which PLC enzymes are modulated in response to receptor occupancy remains unknown. Much evidence (*2, 3*) shows that the occupancy of receptor stimulates PLC activity and this stimulation is mediated through unidentified G proteins. The most convincing evidence presented to date involves the capacity of guanosine triphosphate (GTP) and its nonhydrolyzable analogs to enhance the agonist-mediated inositol trisphosphate (IP_3) formation in permeabilized cells and derived membranes (*22*). The G protein family contains a large number of closely related members, and the function of each member can be modified by pertussis toxin or cholera toxin or both. Efforts to identify the PLC-related G protein by using the two toxins led to observations that are not easily explained; for example, one toxin blocks the receptor-stimulated formation of IP_3 in one cell type although no effect is observed with the same toxin in another cell type (*22*). A possible explanation is that each PLC isozyme might be activated by a distinct G protein; this may provide cells with a capacity to elicit diversified responses to a variety of agonists.

A model involving only the positive activator but no negative modulator for regulation of PLC appears to be inadmissable for the following reasons: All of the purified PLC enzymes are active in the absence of G protein and exhibit a specific activity of 20 to 30 μmol per minute per milligram of protein under optimal conditions (*7–12,*

14). The amount of PIP_2 in brain is 2 to 14 nmol per milligram of protein, depending on the area of brain (*30*), and the total amount of PLC isozymes (mainly PLC-β and PLC-γ) is about 2 μg per milligram of protein in the brain (*10*). Thus, PLC activity in the brain is sufficient to cause complete hydrolysis of PIP_2 in 2 to 20 s even without the receptor-directed stimulation. The calculated lifetime of PIP_2 can be even shorter in striatum and hippocampus where PLC-β and PLC-γ are highly localized (*31*). The futile hydrolysis of PIP_2 in the absence of a negative modulator not only generates an unneeded signal for Ca^{2+} release but also causes a large waste of adenosine triphosphate (ATP) energy, because the resynthesis of PIP_2 from diacylglycerol and inositol requires five equivalents of ATP. In addition, numerous attempts to demonstrate a direct G protein effect on PLC by using various combinations of G proteins (G_o, G_i, and G_t) and purified PLCs (β, γ, and δ) have been unsuccessful in our laboratory, whereas activation of PLC by guanosine 5′-*O*-(3-thiotriphosphate) could be easily demonstrated with crude extracts of brain. It is therefore logical to propose a model in which PLC activity remains repressed by an inhibitory factor and becomes activated only when a G protein relieves the inhibitory effect on occupancy of the receptor by a Ca^{2+}-mobilizing ligand.

Further support for the possible involvement of negative regulatory protein comes from the comparison of the PLC amino acid sequence with several seemingly unrelated proteins. A significant sequence similarity between PLC-γ and nonreceptor protein tyrosine kinases has been described (*26, 27*). Figure 1 shows three clustered regions of similarity with the *src*-encoded family of tyrosine kinases, and these were designated A, B, and C. In the case of PLC-γ, the A, B, and C regions are located between the X and Y domains and the B and C regions are duplicated. In the *src*-related tyrosine kinases, there is only one each of A, B, and C, and the A region is transposed from its relative position in PLC-γ to the COOH-terminal side. It has already been shown that the domain encompassing B and C sequences is located in the noncatalytic domains of tyrosine kinase and this domain has a regulatory role perhaps in directing specific interactions with some cellular components (*32*). A novel viral oncogene, *crk*, which encodes a *gag*-fusion polypeptide $p47^{gag\text{-}crk}$ of 47 kD was isolated from avian sarcoma virus CT10 (*33*). The *crk* gene product has no amino acid sequence similarity with a tyrosine kinase catalytic domain, but contains the A, B, and C regions. The arrangement of the three *crk*-encoded regions has more similarity to the arrangement found in PLC-γ than to the tyrosine kinases, because the *crk*-B and *crk*-C regions precede the *crk*-A region. The defective *crk* gene product does not contain a tyrosine kinase catalytic domain. Nevertheless, as observed with several other transforming retroviruses, CT10 induced an increase in protein phosphotyrosine content in infected target cells (*33*). According to Mayer *et al.* (*33*), one explanation is that $p47^{gag\text{-}crk}$ functions to titrate out negative factors, leading to increased tyrosine kinase activity. The same negative regulatory factors are likely to modulate the activity of PLC-γ, which also contains the putative regulatory domains, A, B, and C. Stahl *et al.* (*26*) noted that the negative regulatory factors are unlikely to be G proteins, which have not been shown to interact with cytoplasmic tyrosine kinases.

Two more proteins were recently added to the list of proteins that might share the same regulatory properties of PLC-γ. The guanosine triphosphatase (GTPase)–activating protein (GAP) that stimulates the GTPase activity of *ras* p21 contains duplicated B- and C-like clusters similar to those of PLC-γ (*34*), whereas the A-like region is found in the α subunit of spectrin (*35*). The separate appearance of a B-C domain and an A domain suggests that these domains could have independent functions. The separate appearance was also observed in the product of c-*fps*, one of the nonreceptor tyrosine kinases that contains only a B-C domain but no A domain.

Among the four PLC isozymes, only PLC-γ contains the A, B, and C domains; the other three enzymes have neither A nor B-C domains. This result suggests that there are different means of regulation for the isozymes. Thus, different modes of modulation may account in part for the diversity of responses observed in different tissues and individual cell types to a variety of external stimuli. Consistent with this notion are results indicating that PLC isozymes are expressed differently between and within tissues and in individual cells and during development (*10, 31*).

Our immunohistochemical study of the rat brain demonstrated the different distribution pattern of PLC isozymes (*31, 36*). PLC-γ immunoreactivity appeared to be relatively ubiquitous, staining essentially all neurons in the brain. PLC-β immunoreactivity also was localized in neurons but displayed a more restricted pattern of distribution. The densest labeling was shown in the neurons in the striatum, hippocampus, and dentate gyrus, and in certain thalamic nuclei, particularly the thalamic reticular nucleus (*31*). In contrast to the neuronal distribution of PLC-β and PLC-γ immunoreactivity, PLC-δ immunoreactivity appeared to specifically label astroglia (*36*).

Regulation of PLC Activity Through Protein Phosphorylation

There is evidence that the protein phosphorylation is involved in both the negative and positive regulation of PLC activity. Treatment of intact cells with the tumor-promoting phorbol esters [activators of protein kinase C (PKC)] decreases the agonist-induced PIP_2 hydrolysis and Ca^{2+} mobilization (*37*). These decreases may be due to phosphorylation of the GTP-binding protein (*38*) or of PLC by PKC. Since diacylglycerol, a product of phosphoinositide hydrolysis, is an endogenous activator of PKC, the PKC-dependent inhibition of PIP_2 hydrolysis might represent a feedback regulation of PLC activity. Phosphorylation of PLC by PKC has been demonstrated in vivo (PLC-α) (*11*) and in vitro (PLC-α, PLC-β, PLC-γ, and PLC-δ) (*39*). However, the effect that phosphorylation of PLC has on signal transduction could not be demonstrated directly because no changes in the catalytic activity could be demonstrated in vitro with any of the three PLC isozymes (β, γ, and δ) as a consequence of the introduction of one to two orthophosphates per PLC molecule (*21*). An explanation for this is that the PKC-dependent phosphorylation causes inhibition of PLC activity in a manner dependent on other modulatory protein factors that are not present in the in vitro system. For example, phosphorylated PLC may be incapable of interacting with the putative positive regulatory factor, G protein, thus remaining inactive because of its association with a negative modulatory protein factor.

Cyclic AMP may also exert an inhibitory effect on the formation of inositol phosphates. For example, treatment of certain cells and tissues with cAMP-elevating agents such as dibutyryl cAMP, theophylline, prostaglandin E_1 and E_2, or prostacyclin blocked the phosphoinositide breakdown (*40*). The mechanism by which cAMP blocks phosphoinositide turnover is unknown. Direct phosphorylation of PLC by a cAMP-dependent kinase is a possibility.

Evidence that protein phosphorylation is involved in the regulation of PLC comes from studies with a growth factor. In contrast to phorbol esters and cAMP, treatment of certain cells with growth factors such as the epidermal growth factor (EGF) and the platelet-derived growth factor initiates the rapid formation of IP_3 in the absence of extracellular Ca^{2+} (*41*). Most growth factors bind to receptors that have intrinsic tyrosine kinase activity. Recently, Wahl *et al.* (*42*) isolated proteins that contained phosphotyrosine from

extracts of EGF-stimulated A-431 cells by means of an antibody to phosphotyrosine and showed that PLC activity is highly enriched in the isolated fraction. This finding suggested that either PLC or a tightly associated protein is an exogenous substrate for EGF-stimulated tyrosine phosphorylation and that this tyrosine phosphorylation has functional consequences that are related to PIP_2 hydrolysis. Subsequently, Wahl *et al.* (*43*) obtained evidence that EGF receptor kinase directly phosphorylates PLC-γ on tyrosine when antibodies to PLC-β, PLC-γ, and PLC-δ are used. When A-431 cells were metabolically labeled in media containing [^{32}P]orthophosphate and then treated with EGF, only PLC-γ was precipitated by antibody. Phosphoamino acid analysis of the precipitated PLC-γ from control and EGF-treated cells demonstrated a 6.5-fold growth factor–dependent increase in the amount of phosphotyrosine. Like EGF, both bradykinin and exogenous ATP also stimulate the rapid formation of IP_3 in A-431 cells. However, only EGF increased the amount of phosphotyrosine, suggesting that bradykinin and ATP stimulate PLC activity by a mechanism independent of tyrosine phosphorylation.

Perspectives

The activation of many cellular functions and the control of cell proliferation depend on the PIP_2 hydrolysis, cAMP formation, and protein tyrosine phosphorylation. There is increasing evidence for a physiological interaction between these receptor-dependent signaling pathways. In addition, the two PIP_2-derived messengers, diacylglycerol and IP_3, are each metabolized further to produce several compounds that may be additional new messengers. Diacylglycerol produces a series of biologically active arachidonic acid metabolites, and IP_3 is phosphorylated to form several inositol polyphosphates. One idea is that the bifurcated cascade reactions constitute positive forward and negative feedback control over the PLC that initiates the cascade.

Specialized function of tissues and cells may require different modes of PLC regulation. The stimulation of certain receptors may result in activating a specific PLC isozyme. The fact that PLC enzymes are unusually large compared with other phospholipases such as phospholipase A_2 (12 to 18 kD) and highly variable (ranging from 65 to 15 kD) is consistent with the notion that large portions of PLC enzymes are reserved for specific regulatory functions.

Future work should include identification of protein factors that interact with PLC and uncovering the possible role of protein phosphorylation in the modulation of a specific PLC. Because specific antibodies are available, it should be possible to precipitate the complexes containing regulatory factors together with a PLC and quantitate the associated phosphate. It may also be possible to selectively inhibit PLC enzymes by microinjecting these antibodies. Moreover, the availability of PLC cDNAs may allow the expression of a PLC together with appropriate membrane receptors and G proteins. This methodology will permit evaluation of functional coupling.

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