Lipids as hormones and second messengers

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Some lipids act as hormones to induce responses in target tissues whereas others influence the behavior of cell-surface receptors and/or serve as second messengers; i.e. they are formed as part of the cellular response to extracellular agonists, and directly affect protein kinases, ion channels and other systems that govern cell behavior. Our understanding of these functions on a structural level is improving, but this is still a relatively new, and an extremely complex, area of investigation.

Introduction

Lipids participate in cellular structure and function at essentially all levels, which probably accounts for the diversity of the lipid classes and subtypes. As stated by Thudichum [1] over a century ago, 'Their physical properties are, viewed from a teleological point of standing, eminently adapted to their functions.' This has proven to be true at at least three levels. Firstly, the molecular features of lipids allow them to define membranes, lipoproteins and other biological structures, as discussed elsewhere in this issue by Reiss-Husson (pp 506–509). Secondly, many lipids also embody structural domains that interact with receptors, enzymes and other components of cell membranes as well as with extrinsic cytoskeletal proteins, antibodies, microbial toxins, etc. (some aspects of this topic are discussed in this issue by Ferguson, pp 522–529). Finally, a growing number of lipids can be converted to products that are highly bioactive as hormones and lipid second messengers. When one considers the iterative fine-tuning that proteins have undergone over the course of evolution, it should not be surprising that the lipid components of biological membranes have also evolved to exhibit similar sophisticated properties.

Overview of lipid hormones and second-messenger systems

As shown in Fig. 1, there are a large number of compounds with proposed functions as lipid second messengers, and we probably know only a fraction of the intracellular systems that are controlled by these types of mechanisms. In most instances, the formation of the lipid hormones (e.g. platelet-activating factor, the eicosanoids, etc.) and lipid second messengers (e.g. diacylglycerols (DAGs), phosphatidic acid (PA), lysophospholipids, ceramides, etc.) proceeds from the cleavage of more complex membrane lipids by phospholipases [2••] that have been activated by the relevant agonist. Nearly all of the possible products of these cleavage reactions (i.e. phospholipid headgroups, lysophospholipids, DAGs, PA and fatty acids) have been implicated in signal transduction. For example, release of lysophospholipids and fatty acids via phospholipase A₂ provides compounds that serve as precursors for platelet-activating factor, prostaglandins, leukotrienes, thromboxanes and other eicosanoids, and as possible modulators of the activity of protein kinase C (PKC). Phospholipases C and D produce DAGs, alkylacylglycerols and PA from diverse phospholipid precursors (including phosphatidylinositol (Pls), phosphatidylcholines (PCs) and PI glycan-linked proteins inter alia). These products can have multiple intracellular targets, such as the activation of PKC by DAGs and the stimulation of Ca²⁺ release from intracellular stores by inositol triphosphate, as shown in Fig. 1. Bioactive lipids can be made in multiple intracellular sites; in Fig. 1, this is illustrated by the formation of steroid hormones but this can also occur, at least theoretically, for most of the other types of lipid second messengers. In addition to these phospholipases for glycerolipids, the hydrolysis of sphingomyelin to ceramides has recently been proposed as another lipid second-messenger cycle [2••, 3]. Other hydrolysis products of sphingolipids (e.g. sphingosine and some lysosphingolipids) are potent inhibitors of PKC [4•], Na⁺/K⁺-ATPase, PA phosphohydrolase, and other phospholipases, as well as activators of the epidermal growth factor receptor; hence, the hydrolysis of sphingolipids to bioactive constituents may prove to be widespread and important [5•].

The multiplicity of these pathways, and the fact that most of the enzymes, substrates and products are membrane-associated, make it difficult to understand these systems on a molecular level. Nonetheless, recent findings have provided a more detailed picture of the interactions between lipid second messengers and their targets, and some of these lessons will be discussed using PKC as a model.

Abbreviations

DAG—diacylglycerol; PA—phosphatidic acid; PC—phosphatidylcholine; PG—phosphatidylglycerol; PKC—protein kinase C; PI—phosphatidylinositol; PMA—phorbol 12-myristate,13-acetate; PS—phosphatidylserine.
Protein kinase C as a model for lipid modulation of a signal-transduction system

Protein kinase C is actually a family of enzymes, therefore caution is to be recommended in making generalizing statements about this system. Nonetheless, the studies reported thus far illustrate many structural considerations that should be pertinent to this and other lipid-modulated signal-transduction systems [6**]. Firstly, the activity is affected both by phospholipids (phosphatidylyserine (PS)) and by phospholipase cleavage products acting as activators (DAGs and unsaturated fatty acids) and inhibitors. Secondly, the phorbol-ester-binding domain (regarded as the lipid-activating domain of PKC) is located in the amino-terminal region — hence, activation is thought to involve a conformation change that displaces a pseudosubstrate from the active site. Thirdly, PKC occurs in multiple intracellular sites so that the localized formation of activators (and inhibitors) and enzyme translocation may be important factors in the biological responses. Finally, PKC activity is also influenced by other factors such as the availability of Ca\(^2^+\), the extent of oxidation of key sulphydryl groups, the conformation of PKC upon insertion into membranes (some forms are less sensitive to lipid modulators), down-regulation by proteases, and many other factors.

Fig. 1. Overview of hormone and signal-transduction pathways involving lipid mediators. Binding of stimuli (hormones, growth and differentiation factors, etc.) to membrane receptors triggers a sequence of events that usually involves cleavage of a membrane lipid to a bioactive fragment or derivative. Examples of the lipases that are involved in these events are as follows. Phospholipase A\(_2\) (PLA\(_2\)) releases a (usually polyunsaturated) fatty acid (FA), which may proceed to other bioactive products such as prostaglandins, etc., from the 2-position of a phosphoglycerolipid; in some cells, lysoalkylphosphatidylycholine (lysoalkyl-PC) undergoes reacylation to produce platelet-activating factors (PAF). Phospholipase C (PLC) cleaves phosphoglycerolipids (such as phosphatidylinositol 4,5-bisphosphate, PIP\(_2\)) to diacylglycerol (DAG) or 1-alkyl- or 1-alkenyl-2-acylglycerols (EAG); DAG activates protein kinase C (PKC) and EAG may activate or inhibit this enzyme. When the substrate is PIP\(_2\), release of inositol triphosphate (IP\(_3\)) increases cytosolic Ca\(^2^+\). Phospholipase D (PLD) cleaves phosphoglycerolipids to phosphatidic acid (PA) which may act directly, or be converted to DAG to stimulate cell responses. Other classes of PLC and PLD act on phosphatidylinositol-glycan-linked proteins to release the protein and DAG or EAG. Membranes also contain sphingolipid (SL) hydrolases, such as sphingomyelinase and ceramidase that can liberate ceramide (Cer) and/or sphingosine (So). Other signal transduction elements in cells include the enzymes that hydrolyse cholesterol esters to cholesterol then to various steroid hormones. In some instances, the lipids act on systems in the same cell (i.e., are second messengers); in others (which have not been distinguished for simplicity), they act on targets elsewhere and are termed hormones. Other abbreviations: PS, phosphatidylycerine.
Structure–function studies of lipid modulators of PKC have adopted two broad approaches. The first has compared the structures of analogs of phorbol esters, DAGs and other potent activators (and inhibitors) of PKC. A major conclusion of these studies has been that DAGs overlay well with phorbol 12-myristate,13-acetate (PMA), with properly orientated oxygen atoms located at sites corresponding to C-4, C-9 and C-20 of PMA, and a hydrophobic component located in the proper region of space, i.e. R and R' of PMA and DAG respectively [7,8], as illustrated in Fig. 2. By reducing the alkyt-chain length of phorbol esters (to phorbol dibutyrate), it is possible to measure binding to PKC directly, and KdS of the nanomolar order are typically obtained. A similar strategy has led to the development of short-chain analogs of DAGs (1-oleoyl,2-acetylglycerol and, more usefully, 1,2-dioctanoylglycerol) as more water-soluble activators of PKC [9]. However, attempts to mimic the potent activation and binding by phorbol esters with glyceride-type molecules have, thus far, been fairly unsuccessful, indicating that the interactions between phorbol esters and PKC go beyond the molecular features of DAGs that are important for biological activity [10].

Fig. 2. Comparison of the presumed bioactive conformation of a diacylglycerol (DAG) with phorbol 12-myristate,13-acetate (PMA).

The second approach has been to elucidate the molecular details of PKC activation by lipid mediators encountered under more physiological conditions. This is much more complicated because it requires knowledge of not only the structural features of the lipids (e.g. stereochemistry, alkyt-chain composition, etc.) but also of the physical state of these molecules. The use of mixed micelles by Bell and colleagues [6••] has simplified these analyses considerably. Figure 3 attempts to illustrate some of the features that are important in understanding PKC activation at this level.

Structural requirements of lipid modulators of protein kinase C

A series of elegant investigations have identified the structural specificity of PKC for DAGs and PSs (reviewed in [6••]). Activation requires ester-linked fatty acids at positions 1 and 2 of sn-1,2-diacylglycerols (1,3- and 2,3-isomers are not active) and a free hydroxyl group at position 3. The nature of the alkyt chains is less important, and shorter-chain DAGs will activate if they are sufficiently hydrophobic to be membrane bound [9]; for this reason, a fairly close and specific interaction between PKC and the glycerol moiety has been depicted in Fig. 3.

The major structural requirements for PS binding to PKC appears to be the t-serine headgroup as other naturally occurring phospholipids, and synthetic phospholipids made from serine analogs, are not as effective [6••,11]. In contrast, there is little specificity inherent to the glycerol backbone or the fatty acyl chains, and 1,3-diacyl-PS is able to activate PKC despite its considerable deviation from the usual 1,2-diacyl configuration [6••,11]. The exact stoichiometry of the interaction between PKC and PS is not known; however, on the basis of the co-operativity of activation in a mixed micelle system [12] and similar studies with vesicles [13], at least four PSs appear to be involved. Hence, several interactions between PKC and the headgroups of PSs have been depicted in Fig. 3. Flexibility has been left in the diagram in this region, nonetheless, because some activation can be achieved by other acidic lipids [6••,14•]. In the case of PKC activation by PI 4,5-bisphosphate, the effect was to reduce the concentration of PS required for activation [14•]. This may indicate that there are a few sites that are highly specific for PS and others that accommodate other acidic lipids more readily. Not depicted in Fig. 3 is the ability of certain lipids, especially unsaturated fatty acids [15•] but perhaps other lipids as well [16•], to interact with cytosolic PKC.

Physical properties of lipids related to their effects on protein kinase C

What is the actual nature of the interactions between PKC and these lipid activators, Ca2+, the protein substrate, and other factors that can influence the activity of this enzyme? One difficulty in envisioning the lipid modulation of PKC and other membrane-associated processes is the necessity for diagrammatically depicting them in two dimensions and in a static state. Membrane phospholipids typically have a high degree of mobility (in-plane exchange rates for phospholipids are of the order of 107 s−1 [17••]) and dipalmitoyl-PC, for example, has been estimated to have 436,755 rotational isomeric configurations [18•]. A 'snapshot' of the membrane such as that shown in Fig. 3 clearly poses some impediment to fully appreciating the behavior of these molecules. The membrane surface that modulates the activity of PKC should be viewed, to paraphrase Roux and Bloom [19••],
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Fig. 3. Hypothetical depiction of the interactions of protein kinase C with Ca\(^{2+}\), phosphatidylserine (PS) and di-acylglycerol (DAG) at the membrane interface. The lipid components are represented using space-filling models which were individually subjected to energy minimization calculations. The lighter shaded area at the membrane interface symbolizes waters of hydration.

It appears that the initial interaction of PKC with the membrane is mediated through its binding to PS and Ca\(^{2+}\) [29*,30*], and that the Ca\(^{2+}\)-binding site(s) are generated at the interface between PKC and the membrane [30*]. Calcium is well known to form a complex with PS [31,32*]; hence, a Ca\(^{2+}\)-binding site may be created by a mechanism like that depicted in Fig. 3. It is thought that after the enzyme has bound PS and Ca\(^{2+}\), it binds DAG (or phorbol esters) to become fully active [33]. Phosphatidylserine affects the specificity of PKC such that substrate phosphorylation is favored over autophosphorylation [34*], presumably through reorientation of amino acid groups coupled to the catalytic site of the enzyme. The binding of PKC to membrane induces an increase in surface pressure [29*] that can be interpreted as a reorientation of the phospholipids and/or possible insertion of a protein domain into the membrane (Fig. 3). The binding of PKC to PS is also expected to induce a reorientation of the lipid, as indicated by model studies with amphipathic peptides [35*].

Elegant studies of the physical behavior of the 1,2-DAG activators of PKC [36*] indicate that the sn-2 carbonyl experiences a significantly greater degree of hydrogen-bonding than the sn-1 carbonyl at the aqueous interface, as indicated in Fig. 3. Furthermore, the transbilayer movement of 1,2-dilauroyl-sn-glycerol is extremely fast (t\(_{1/2}\) is \(~10\) ms).

In addition to these physical considerations, it should be remembered that biological membranes are undergoing constant metabolism (albeit under careful metabolic control) and vesicular movement (it is estimated that the equivalent of the entire surface of many cells is internalized every 20–30 min). There appear to be discrepancies in the ability of DAGs liberated from different phos-
phoglycerolipids to activate PKC, and some of these may reflect intracellular compartmentalization [37*]. Furthermore, the asymmetry of PS in plasma membranes appears to be maintained by ATP-dependent 'aminophospholipid translocase(s)' [17**]. These issues are especially pertinent to PKC because many of the biological processes regulated by this enzyme involve some form of membrane movement and remodelling.

References and recommended reading

Papers of special interest, published within the annual period of review, have been highlighted as:

• of interest
• of outstanding interest


A concise up-to-date review of the properties of phospholipase A₂, C and D, sphingomyelinase and their products (arachidonic acid, alkytyetherlysophosphatidylcholine, DAG, PA and ceramide).


A study of the effects of 1α,25-dihydroxyvitamin D₃ on sphingomyelin turnover to ceramide, and the induction of 6-hydroxy cell differentiation by a ceramide analog. These studies indicate that ceramides may be formed from sphingomyelin and act as another class of lipid second messenger to mediate the action of this, and possibly other, hormones.


A review of the properties of sphingolipids, sphingolipid metabolism and possible roles of sphingosine and other more complex sphingolipids in regulating PKC and various aspects of cell behavior.


A minireview of the structure–function relationships between PKC and lipid activators and inhibitors. A scheme for the possible interactions between the enzyme, its pseudosubstrate domain and the phospholipid, DAG and Ca²⁺ binding sites is proposed.


PKC activation is investigated using a Triton X-100 mixed-micelle assay. Phosphatidylinositol 4,5-bisphosphate activates PKC in a Ca²⁺-, and PS-dependent manner, reducing the concentration of PS required for activation. This may be important in vivo if the availability of PS for PKC is limiting.


Sodium oleate preferentially activates soluble PKC relative to membrane-associated enzyme by a mechanism that is distinct from activation by DAGs or phorbol esters. The activation exhibits mild cooperative behavior which suggests that Ca²⁺(oleate)₂ may be the active species.


α-Tocopherol stimulates phorbol dibutyrate binding by smooth muscle cells and inhibits the translocation, activation, and the phorbol-ester-induced down-regulation of PKC. The results indicate that α-tocopherol may interact with the cytosolic form of PKC, and that this may account for the effects of vitamin E on cell proliferation.


A review of the asymmetric distribution of phospholipids in eukaryotic cell membranes, the ways that transbilayer asymmetry can be established, and the possible biological functions of these processes.


A detailed model for the structure and dynamics of the interior of the lipid bilayer.


An analysis of cation binding to 1-palmitoyl-2-oleoyl-PC and PS. The 2H NMR spectra suggest that Ca²⁺ is bound in at least two steps, the first occurring at a stoichiometry of 0.5 Ca²⁺ per PS. Roux and Bloom interpret the data as reflecting conformational changes in the phospholipid headgroups induced by an electric field resulting from the charges of the membrane-bound cations, with Ca²⁺ and Mg²⁺ more deeply buried in the membrane than Na⁺ or K⁺.


At neutral pH, dioleoyl-PS adopts a bilayer configuration with ordering of the fatty acyl chain that is only slightly increased over that of dioleoyl-PC. At lower pH (pH < 6), electrostatic repulsion between the PS headgroups is reduced, intermolecular hydrogen-bonding interactions are stronger, and the behavior of PS begins to resemble that of phosphatidylethanolamine.

22. SPAOER T, CLARK SB, GANTZ DL, HAMILTON JA, SMALL DM: The Ionization and Distribution Behavior of Oleic Acid in Chylomicrons and Chylomicron-like Emulsion Particles
The transbilayer transport of PA is measured using large unilamellar vesicles prepared from dioleoyl-PC and PA and with detection of the acidic lipid based on its effect on fluorescence of 2-(p-toluidinyl)naphthalene-6-sulfonic acid. Phosphatidic acid crossed the bilayer most rapidly in the neutral (protonated) form with a half-time of 4.1 min at 45°C and an activation energy of 20 kcal mol⁻¹.


Two fluorescent pH-sensitive probes are used to measure changes in the acidic lipid content at 45°C and an activation energy of 28 kcal mol⁻¹.


Two fluorescent pH-sensitive probes are used to measure changes in lipid packing and the sensitivity of the surface pH of micelles and membranes to changes in bulk pH. The surface pH of micelles is very sensitive to bulk pH, but the pH of PC or PA bilayers changes little. High-pressure spectroscopy is used to determine the effect of increased chain packing. Pressure causes proton dissociation from the micelles, but has less of an effect in the case of bilayers.


Fluorescence, electron paramagnetic resonance, electrophoretic mobility and ionizing electrode measurements are used to study the effect of PI and P, 4.5 bisphosphate on the electrostatic potential adjacent to bilayer membranes.


The association of PKC with membranes is assessed by the increase in monolayer surface pressure using a mixed phospholipid film. The addition of Ca²⁺ results in an increase in the penetration of the kinase (DAG and phorbol esters have no effect); hence, Ca²⁺ may play a major role in the translocation of PKC to membranes.


An analysis of Ca²⁺ binding by PKC using equilibrium dialysis. Free PKC binds virtually no Ca²⁺, but at least eight Ca²⁺ ions are bound in the presence of acidic phospholipids, after correction for Ca²⁺ binding by the phospholipids alone. These findings suggest that the Ca²⁺-binding sites 'may be generated at the interface between PKC and the membrane'.


An analysis of high-affinity Ca²⁺ binding by PS in PS/PC multilayers. From these data, the authors calculate the excess partial free energy to transfer 1-palmitoyl-2-oleoyl-PS from PS to PC as ~0.7 kcal mol⁻¹, and suggest that this arises in part from the transfer of negatively charged PS from an environment of negative charges to one of zwitterions.


An analysis of PKC activity using small unilamellar vesicles of varying composition. Increasing the PS concentration (and the negative surface charge) favors substrate phosphorylation over autophosphorylation, whereas DAG increases both activities equally. Hence, PS affects the specificity of PKC toward different substrates.


Amphiphilic peptides reduce the order parameter of both the dioleoyl-PS headgroup and acyl chains. The response of PS to the divalent peptides fits the electrostatic concept, i.e. the effective charge experienced by PS is related to the distance between the charges. A divalent peptide with a distance between the charges of ~10 Ǻ will have an effect similar to NaCl, which has a Debye length of 100 nm. Disruption of the phospholipid acyl chain order appears to occur with cations that are localized in the lipid-water interface and, in certain cases, where there is a collapse of the integrity of the vesicles.


The behavior of 1,2-dilauroyl-sn-glycerol in PC is analysed by 31P and 1H NMR spectroscopy and other physical techniques. The NMR results indicate that the sn-2 carbonyl experiences a significantly greater degree of hydrogen-binding than the sn-1 carbonyl at the aqueous interface. Hence, the orientation of the glycerol moiety is more like that of PC, with the sn-2 carbonyl more accessible to solvent interactions. The transbilayer movement of 1,2-dilauroyl-sn-glycerol is extremely fast (t₁/₂ of ~10 ms).


Treatment of ICG fibroblasts with different amounts of α-thrombin stimulates differential formation of DAGs from PI 4.5-bisphosphate and PC. The results indicate that DAGs from the former, but not the latter, are able to activate PKC in this system.

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