# Phospholipids as dynamic participants in biological processes

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**Abstract** Phospholipids are described as active biological molecules. Three distinctly different roles are examined. The first centers on protein-lipid interactions and the lipid requirement expressed by certain enzymes. This category is illustrated by two soluble proteins of the blood coagulation scheme, Factor IXa and Factor Xa, and by an integral membrane protein, the ($Ca^{2+}$ + $Mg^{2+}$)-ATPase of human erythrocytes. The next two examples depict phospholipids as active participants in membrane-mediated events. In the first of these, termed the phosphoinositide effect, a phospholipid becomes a substrate during membrane signaling, and its products presumably act as second messengers. In the second example, a phospholipid is a signal that, among other reactions, induces the phosphoinositide effect. Here, the phospholipid (platelet activating factor) serves as a lipid chemical mediator. These examples show that phospholipids behave not only as structural molecules but also as dynamic, functionally important components of cells.—**Hanahan, D. J., and D. R. Nelson.** Phospholipids as dynamic participants in biological processes. *J. Lipid Res.* 1984. **25:** 1528–1535.

**Supplementary key words** protein-lipid interactions • lipid requirement • blood coagulation • human erythrocyte ($Ca^{2+}$ + $Mg^{2+}$)-ATPase • phosphoinositide effect • phosphatidylinositol-4,5-bisphosphate • platelet activating factor

The complex array of phospholipids present in membranes has titillated the scientific senses of lipid biochemists and membranologists for many years. Interest has spanned the gamut from isolation and structure proof studies on these lipids to their organization in the membrane and thence to possible involvement in membrane-mediated events. The problems inherent in the study of membrane processes as they relate to the phospholipids have been well documented in several reviews (1–4) and it is not the intention here to duplicate these efforts. This review will center, rather, on two major areas. The first addresses protein-lipid interactions and the direct physical effects phospholipids can have on enzymatic reactions. The second examines phospholipids as participants in complex processes, where they serve both as substrates and chemical mediators. Four examples have been chosen to illustrate some unique physical and biochemical properties of phospholipids. This sampling emphasizes the importance of phospholipids as dynamic cellular and extracellular components and not just as the building blocks of biological membranes.

## I. PHYSICAL INTERACTIONS

Two protein-lipid interactions will be explored here, both illustrating the importance of physical interactions between glycerophospholipids and enzyme systems. The first will describe glycerophospholipid effects on the blood coagulation system, particularly the proteolysis of prothrombin by Factor Xa and the activation of Factor X by Factor IXa. The second will examine the role of lipids in the function of a membrane transport protein: the ($Ca^{2+}$ + $Mg^{2+}$)-ATPase of human erythrocytes. These are examples of phospholipids acting as enhancers of enzymatic activity in both a solule and membrane bound system.

### A. Proteolysis of prothrombin: the prothrombinase complex

Perhaps the earliest evidence for the importance of phospholipids in influencing specific reactions in the blood coagulation pathway came from the studies of Papahadjopoulos and Hanahan (5) in 1964. In this investigation, attention was directed at definition of the interactions between components of the prothrombinase complex that led to the production of thrombin. The components included the serine protease, Factor Xa, which is generated from Factor X, calcium, Factor V, and phospholipid. Various combinations of these components were chromatographed on Sephadex G-200. From the results it was evident that the most potent form of this activator was one in which Factor Xa, Factor V, calcium, and phospholipid were present. A mixture of all these components behaved like a large molecular weight complex and eluted in the void volume of Sephadex G-200. This complex had potent protease activity towards prothrombin. It could be dissociated by treatment with EDTA into a Factor V·phospholipid component, Factor Xa, and calcium, all of which were



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well separated from each other by gel filtration. Studies prior to this one by Papahadjopoulos, Hougie, and Hanahan (6) had shown quite convincingly that glycerophospholipids could have a significant influence on the above reaction, but only those lipids bearing a negative charge were effective. The charge requirement could be easily achieved with the proper combination of phosphatidylcholine and phosphatidylserine. Later, Barton, Jackson, and Hanahan (7) reported that the prothrombin activator complex, or prothrombinase, could be separated by passing it through a Sephadex G-200 column, in the absence of added calcium. The complex split into a Factor V·phospholipid complex, which eluted in the void volume, and Factor Xa, which eluted with a Ve/Vo of 2.3. Though the separated Factor Xa showed weak or limited activity towards prothrombin in the presence of calcium ion, the Factor V·phospholipid complex alone showed none. However, mixing of these two fractions together with calcium ions led to formation of a potent prothrombinase activity which eluted in the void volume of the Sephadex G-200 column (in the presence of calcium ions). These were particularly exciting observations and in the ensuing years, with the advent of highly purified coagulation factors and more sophisticated techniques, a better understanding of the prothrombinase system has emerged. In a series of well-designed experiments Esmon, Owen, and Jackson (8) showed the importance of phospholipid and Factor V on the rate of conversion of prothrombin to thrombin by Factor Xa. These and other observations from their laboratory laid the ground-work for a more refined examination of the prothrombinase complex and its activity by Rosing et al. (9). In effect, these investigators examined the kinetic constants of thrombin formation by various prothrombin-activating mixtures. Only a cursory examination of their data can be undertaken here but, nevertheless, it will show the decided influence of the various components on the prothrombinase activity.

| | $K_m$ μM for Prothrombin | $V_{max}$[a] |
|---|---|---|
| Factor Xa | 131 | 0.61 |
| Factor Xa, $CaCl_2$ | 84 | 0.68 |
| Factor Xa, $CaCl_2$, Factor Va | 34 | 373 |
| Factor Xa, $CaCl_2$, phospholipid[b] | 0.032 | 1.06 |
| Factor Xa, $CaCl_2$, phospholipid,[b] Factor Va | 0.21 | 1919 |

[a] mol thrombin produced · $min^{-1}$ · mol $X_a^{-1}$.
[b] Phospholipid, a mixture of dioleoyl phosphatidylcholine and dioleoyl phosphatidylserine.

This abbreviated examination of the extensive data presented by Rosing et al. (9) does not do full justice to their study but it does serve the immediate purpose of showing the specific effects of the components of the prothrombinase complex on its activity.

## B. Proteolysis of Factor X

A second example of the participation of phospholipids in a blood coagulation reaction is the activation of Factor X in the intrinsic system. Activation of Factor X has been shown under in vitro conditions to involve an interaction of Factor IXa, Factor VIII, calcium, and phospholipids. It is clear that Factor IXa is a protease which alone can attack Factor X to yield Factor Xa, albeit slowly. The general events in this activation step are very similar to those described above for the prothrombinase complex. Thus, Factor VIII is considered to be a high molecular weight co-factor, like Factor V, and forms a tight complex with glycerophospholipids bearing a net negative charge. In a particularly interesting and well-documented study, van Dieijen et al. (10) examined the kinetic behavior of Factor X activation by Factor IXa and the influence of calcium ions, Factor VIIIa (thrombin-activated Factor VIII), and a glycerophospholipid mixture (dioleoyl phosphatidylcholine–dioleoyl phosphatidylserine 3:1). The activation of Factor X using various combinations of these components can be expressed most conveniently as follows.

| Component | $K_m$ μM | $V_{max}$[a] |
|---|---|---|
| Factor IXa + Factor X | 299 | 0.022 |
| Factor IXa + Factor X + 10 mM $CaCl_2$ | 181 | 0.0105 |
| Factor IXa + Factor X + 10 mM $CaCl_2$ + 10 mM phospholipid | 0.058 | 0.0025 |
| Factor IXa + Factor X + 10 mM phospholipid + 10 mM $CaCl_2$ + Factor VIIIa | 0.0063 | 5000 |

[a] mol Xa · $min^{-1}$ · mol $IXa^{-1}$.

These results attest to the dramatic influence of the phospholipid mixture on the $K_m$ for Factor X changing it from 299 μM to 0.058 μM (which is close to the plasma concentration of Factor X). The 200,000-fold increase in the $V_{max}$ of Factor Xa formation is explicitly due to the enhancing effect of Factor VIIIa. Interestingly, Factor VIII is not active in the system but must be "activated" first to Factor VIIIa by thrombin treatment. The evidence supplied by van Dieijen and collaborators (10) supports the formation of a complex that can be considered in a general form as:

$$\text{Factor VIIIa} \cdot \text{glycerophospholipids} - Ca^{2+} - \text{Factor IXa.}$$

The above examples show quite clearly the potent influence of phospholipids, expressly carrying a net negative charge, on the conversion by proteolysis of a protein lacking enzymatic activity to one possessing activity. Undoubtedly these phospholipids behave as membranous surfaces on which the reaction can proceed and, to date, no evidence has been presented to show



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any chemical changes in the phospholipids during these activation processes.

Finally, these reactions, studied with highly purified components in an in vitro system, take on added significance as a result of the recent observations of Bevers, Comfurius, and Zwaal (11). These investigators have shown that human platelets, activated by collagen or thrombin for example, exhibit an increase in their ability to convert prothrombin to thrombin by Factor Xa in the presence of Factor Va and calcium. Similar effects were noted after treatment of the platelets with highly purified phospholipases $A_2$ or sphingomyelinase. Their data supported a reorientation of phosphatidylserine from the inner layer to the outer layer of the lipid bilayer, hence presumably allowing availability of the negatively charged phospholipid at the outer surface of the plasma membrane.

### C. Lipid-protein interactions: human erythrocyte ($Ca^{2+}$ + $Mg^{2+}$)-ATPase

The transport of ions and small molecules across cell membranes is mediated by specific integral membrane proteins. These proteins have transmembranous segments in direct contact with the surrounding bilayer lipids. Thus, the membrane lipids act as a solvent for the hydrophobic portions of these proteins much like water acts as a solvent for their hydrophilic domains. However, protein-solvent interactions are much more diverse in lipid than in water. Such variation is exploited in nature and some membrane proteins have evolved specific lipid requirements. For example, cytochrome c oxidase requires cardiolipin (12) and the $Na^+/K^+$ ATPase must have acidic phospholipids (13). Other transport proteins, such as the sarcoplasmic reticulum calcium ATPase, are nonspecific, requiring only a hydrophobic environment that can be supplied by detergent (13). The human erythrocyte ($Ca^{2+}$ + $Mg^{2+}$)-ATPase is a calcium-transporting membrane protein (14–16), which is intermediate in its lipid requirement (13). As such, it provides a useful example illustrating interactions between lipids and membrane proteins.

The purified human erythrocyte ($Ca^{2+}$ + $Mg^{2+}$)-ATPase is not active in polyoxyethylene detergents such as Triton X-100 or Triton N-101, but activity can be restored by adding phospholipids in detergent–phospholipid mixed micelles (17). From these experiments, acidic phospholipids were found to be more effective than zwitterionic species, indicating that a particular lipid environment must exist around the ATPase for optimal activity. The observation that the enzyme is inactive in Triton detergents, partially active in zwitterionic phospholipids, and most active in acidic phospholipids is consistent with a requirement for net negative charge. The total lack of activity seen with Triton detergents corresponds with a total lack of negative charge. Partial activity observed with phosphatidylcholine may be conferred by the negatively charged phosphoryl group, which is partially screened by the positive choline moiety. Maximal ATPase activity seen with acidic phospholipids corresponds well with a substantial negative charge density around the enzyme. An increase in negative charge around the enzyme might also explain the activation of ATPase seen when small amounts of fatty acids or SDS are added to erythrocyte ghosts (17–20). Phospholipase $A_2$, which generates negatively charged free fatty acids in the membrane, also causes an initial activation (20–22).

An additional phenomenon that favors the importance of negative charge is activation of the ($Ca^{2+}$ + $Mg^{2+}$)-ATPase by calmodulin. ATPase in ghosts or ATPase reconstituted into phosphatidylcholine vesicles can be activated by calmodulin, but purified ATPase in phosphatidylserine vesicles cannot because it is already maximally active (17). Calmodulin is very acidic, with a net charge of −16 at neutral pH with four bound calcium ions. When bound either to ATPase in ghosts or to reconstituted ATPase in phosphatidylcholine vesicles, calmodulin can substantially increase the net negative charge around the enzyme. This effect is not so pronounced when calmodulin binds to ATPase in phosphatidylserine vesicles, since a large negative charge has already been supplied by the phosphatidylserine.

Undoubtedly, negative charge is necessary for optimal ATPase activity, but it is not the only requirement. Experiments with phospholipases have shown that the fatty acyl linkages to the glycerol backbone are also important. When ghosts were treated with phospholipase C, the ATPase was inactivated but activity could be restored by adding back any glycerophospholipid, including lysophosphatidylcholine (23). Sphingomyelin, which in many ways resembles phosphatidylcholine, could not reactivate the ATPase. This failure strongly suggests that the structure of the lipids around the first and second carbon of the glycerol moiety is very important for the function of the ATPase molecule. Since lysophosphatidylcholine was able to reactivate the enzyme, the acyl linkage at the first carbon may be more significant than that at the second carbon, or the oxygen at the second carbon may be required. These structural effects may provide an alternative explanation for the inactivity of the purified enzyme in Triton detergents.

Other experiments with phospholipases have localized essential lipid-ATPase interactions at the inner leaflet of the erythrocyte membrane. Phospholipase $A_2$ and sphingomyelinase were used to totally digest the outer leaflet of the erythrocyte membrane of intact erythrocytes without affecting ATPase activity (23). But when ghosts were treated in a similar manner, the ATPase



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activity decreased as the inner leaflet phospholipids were degraded. The relationship was linear with the percent activity remaining being equal to the percent of intact phospholipids in the inner leaflet (23). The outer leaflet lipids, though important for maintaining the integrity of the membrane, appear to have no role in calcium ATPase function.

Recent results from our laboratory indicate that a close association between the ATPase and many phospholipid molecules is necessary for maximal ATPase activity. In experiments where increasing amounts of phosphatidylserine were added to purified ATPase in the form of Triton N-101–phosphatidylserine mixed micelles, maximal activity was achieved at approximately 50 phospholipids per micelle (**Fig. 1**). Since the suggested molecular weight of the ATPase membrane-spanning segment is 33,500 (16), about 30 to 35 phospholipid molecules would be required to cover the surface of a protein cylinder 42 Å in height with this molecular weight. Our results imply that for maximal activity to occur, enough phospholipid must be present to coat the entire hydrophobic surface of the ATPase molecule. The additional phospholipid is probably present in the detergent micelle, away from the ATPase molecule. The number of phospholipids involved reduces the likelihood that a small number of high affinity sites for phospholipid could confer activity. Instead, there appears to be a general requirement for contact with glycerophospholipids over the hydrophobic surface of the protein, especially that part which is normally associated with the inner leaflet of the membrane. This result also tends to eliminate effects of a second or third layer of lipids around the protein since only enough phospholipid is required to form a single layer.

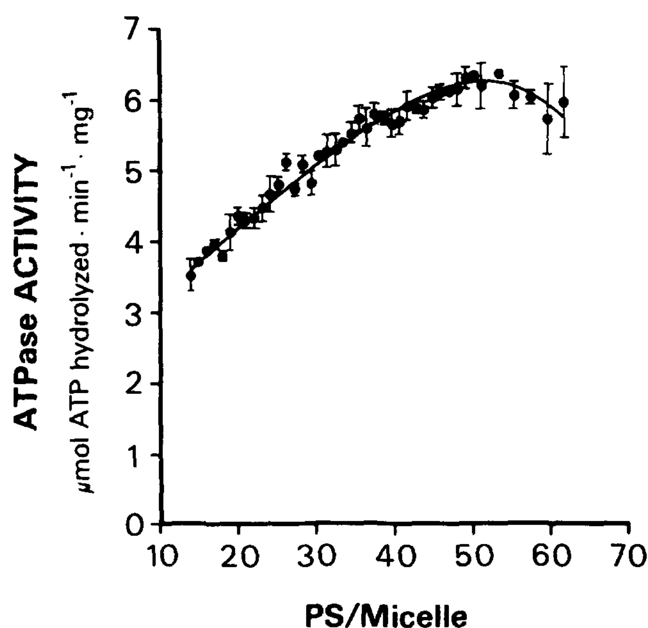


**Fig. 1.** ATPase activity is plotted versus phosphatidylserine (PS) molecules/micelle. All assays contained 60 μg/ml phosphatidylserine. The detergent concentration varied from 178 mM at 62 PS/micelle to 618 μM at 14 PS/micelle. Peak activity occurred near 208 μM Triton N-101. The number of PS/micelle was calculated assuming 100 detergent monomers/micelle and a critical micelle concentration of 89 μM for Triton N-101. Depression of the CMC by phospholipid was taken into account.

In addition to negative charge and the structure around the glycerol backbone, fatty acid chain length and degree of unsaturation are also important for ATPase activity. When synthetic lysophosphatidic acids or lysolecithins with defined fatty acyl groups were added to ghosts, ATPase activity was clearly affected. Both lysophosphatidic acid and lysophosphatidylcholine with an 18:2 fatty acyl chain caused a threefold activation of ATPase activity at $1.6 \times 10^{-4}$ M, while their 18:0 analogues had little or no effect (A. Tokumura, M. H. Mostafa, D. R. Nelson, and D. J. Hanahan, unpublished observations). At least in ghost membranes, the degree of unsaturation was much more important than the nature of the polar head group. Similarly, unsaturated lysophosphatidic acids caused activation which was strongly dependent on chain length with maximal activity occurring at a chain length of 14 carbons. Lysolecithins also affected ATPase activity in a manner dependent on chain length but to a lesser extent than the phosphatidic acids.

Thus, the human erythrocyte ($Ca^{2+}$ + $Mg^{2+}$)-ATPase is very sensitive to its lipid environment. Even though the enzyme has no requirement for a particular phospholipid, it does interact with and respond to every part of the phospholipid molecules that surround it. The charge of the polar head group, the glycerol backbone structure, the fatty acid chain length, and degree of unsaturation all influence calcium ATPase activity. The protein appears to require contact with many phospholipid molecules over its hydrophobic surface and the interactions affecting activity seem to be restricted to the inner leaflet of the membrane. As an example of membrane protein-lipid interactions, the human erythrocyte ($Ca^{2+}$ + $Mg^{2+}$)-ATPase provides a model rich in variety and substance.

## II. METABOLIC INTERACTIONS

While the above section describes the importance of the physical-chemical structure of glycerophospholipids on biological reactions, two examples will now be cited in which metabolic processes involving glycerophospholipids can have equally dramatic effects on cellular reactions. Both of the following examples, the first often termed the "phosphoinositide effect" and the second,



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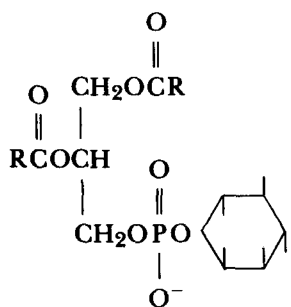
platelet activating factor activity, exhibit important biochemical and physiological effects on intact cells.

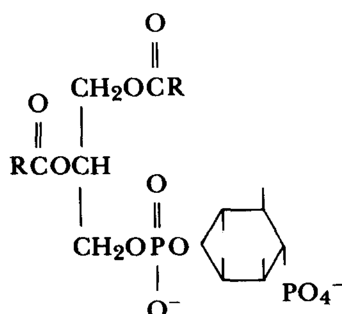
### A. The "phosphoinositide" effect

Thirty years ago, Hokin and Hokin (24) published a now classic paper in which they showed that stimulation of pancreas slices, resulting in secretion of amylases, resulted in a concomitant turnover (measured by $^{32}P_i$ incorporation) of membrane phospholipids. Of considerable interest, phosphatidylinositol (PI) and phosphatidic acid were the phospholipids most active during this process. This type of reaction has been shown to occur in several different tissues and has become the battle cry of investigators interested, for example, in agonist interactions with cells. These reactions are typically rapid, occurring within a 5–30-second time frame. There was a period after the initial flurry of activity based on the report of Hokin and Hokin (24) when this area of study was limited to a relatively few laboratories but the report of Michell (25), on the involvement of the inositol-containing phospholipids in membrane processes such as calcium gating, changed the thinking and direction of the scientific community in a quite significant way. At this point, a brief overview of the "PI" effect will be presented.

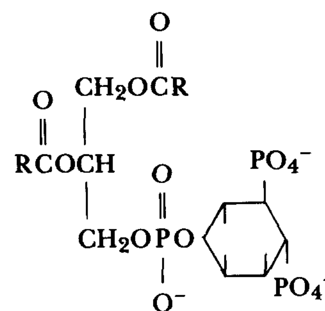
An examination of the chemical nature of the inositol phospholipids in a mammalian cell such as the rabbit platelet shows the following compounds to be present:



Phosphatidylinositol
(PtdIns)



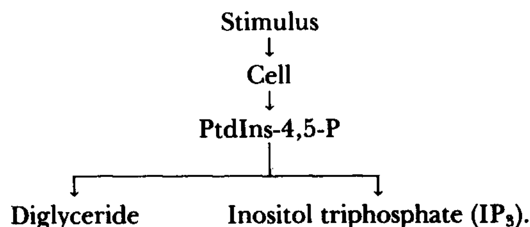
Phosphatidylinositol-4-phosphate
(PtdIns-4-P)



Phosphatidylinositol-4,5-bisphophate
(PtdIns-4,5-$P_2$)

The relative level of these compounds (based on percent of the total inositol lipid fraction) is as follows: phosphatidylinositol, (PtdIns), 81; phosphatidylinositol-4-phosphate (PtdIns-4-P), 11; phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-$P_2$), 8. Using this cell as an example, addition of an agonist, such as platelet activating factor (see next section) causes a very rapid turnover of the PtdIns-4,5-bisphosphate within a 5–10-second period (26). This phenomenon occurs prior to the expression of the biological reaction as monitored by aggregation and by secretion of serotonin.

Current dogma as to the sequence occurring in such membrane reactions can best be described as follows:




Subsequently, these two derivatives are considered to participate in two important reactions. As proposed by Nishizuka (27), diglyceride has a pronounced effect on protein kinase C and the $IP_3$ is considered to exert a major influence on increasing cytoplasmic calcium ions.

This area of study has received an enormous amount of attention and thus the only purpose here is to note that the current wave of enthusiasm among membranologists has lifted inositol triphosphate to an exalted position as a broad-based second messenger. It is only necessary to comment that this field is very exciting and gives impetus to unravelling the mysteries surrounding the many diverse types of phospholipids in membranes.

### B. A biologically active glycerophospholipid: platelet activating factor

Within the past 7 years, an entirely new concept has emerged in the glycerophospholipid arena, that of a



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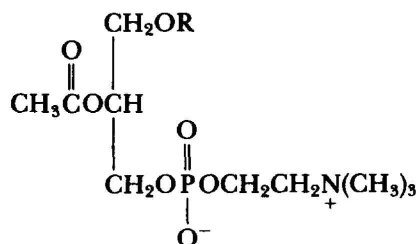
glycerophospholipid that interacts, as an agonist, with a cell yielding very definitive biological reactions (28, 29). This new lipid chemical mediator is 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (AGEPC, often termed platelet activating factor). Its structure is shown below:



This compound also possesses antihypertensive activity (30). It is a unique compound in that it is not found in tissues or cells until they are stimulated. Early studies on its generation in situ centered on the use of rabbits immunized with horseradish peroxidase. If this antigen was infused into these immunized animals, systemic anaphylaxis occurred and AGEPC could be detected shortly thereafter in the blood (31). Interestingly, if the basophils from similarly immunized animals were isolated and assayed for this factor, none could be detected. However, when these isolated cells were incubated with the antigen, platelet activating factor was produced and released.

The lower concentration limit for biological activity of AGEPC, as measured by its ability to initiate aggregation and release of serotonin from washed rabbit platelets, is in the range of $1 \times 10^{-11}$ molar. In addition to affecting platelets, this compound also has pronounced effects on a variety of tissues and cells, such as smooth muscle, liver, lung, and heart causing a wide range of physiological and biochemical changes. It is also active in subhuman primates, in man, rats, and dogs.

Certain features of its chemical structure deserve some mention here. First, the presence of an alkyl ether linkage at carbon 1 of the glycerol is important as is the acetyl moiety at the carbon 2. This latter component is particularly unique since the usual glycerophospholipids found in mammalian cells contain hydrocarbon residues (as esters) 16 to 22 carbons long at these two positions. The phosphocholine head group also appears to be of paramount importance to the biological activity of this compound. To date, no synthetic compound has been produced that has greater biological activity (unpublished observations). In further comments on the importance of the substituents at carbon 1 and carbon 2, the following data illustrate the influence of varying the substituents on the potency (molar) of this lipid chemical mediator (unpublished observations):

| Substituent at Carbon 2[a] | Substituent at Carbon 1 | |
|---|---|---|
| | 1-O-alkyl | 1-O-acyl |
| Acetyl | $1 \times 10^{-10}$ [b] | $1 \times 10^{-7}$ [b] |
| Propionyl | $5 \times 10^{-9}$ | $5 \times 10^{-7}$ |
| Butyryl | $1 \times 10^{-8}$ | Inactive |
| Hexanoyl | $5 \times 10^{-6}$ | Inactive |

[a] Removal of acyl substituent at carbon 2 results in an inactive derivative.
[b] Molar response, based on $[^3H]$serotonin release.

When this AGEPC interacts with a cell, the first recognizable biochemical event is the turnover of the inositol phospholipids (particularly the PtdIns-4,5-$P_2$ as noted above). This action precedes the biological one which occurs within the next 30–60 seconds. Subsequent to the biological action, "excess" AGEPC appears to be metabolically converted to an inactive long chain fatty acyl ether lecithin (32, 33).

## SUMMARY


This review has provided only a few examples of phospholipid participation in membrane-associated processes. Nonetheless, one can assert with reasonable confidence that glycerophospholipids do exert a dynamic influence in and on membranes. Hence, they should not be depicted simply as membrane structural components. Some caution should be exhibited, however, in accepting unequivocally the importance of glycerophospholipids as physical activators of membrane reactions. Most of the evidence cited here in support of physical interactions has been gained from experiments conducted in isolated systems, in which highly purified proteins, such as those of the blood coagulation process, have been shown to be dramatically influenced in their reactivity by the addition of exogenous phospholipids. The same point can be applied to the $(Ca^{2+} + Mg^{2+})$-ATPase system, where a plasma membrane protein, isolated from its normal environment has been shown to be sensitive to added glycerophospholipids. Whether these effects occur in a similar manner in an intact cell system remains a point of conjecture and hence elucidation of the physical effects in situ will have to await more sensitive examination of the interaction of proteins and lipids in the natural environment of the membrane and cell.

While the studies on the physical influence of lipids on cellular enzyme systems are most interesting and provocative, the finding of a biologically active glycerophospholipid, often called platelet activating factor, has added a new dimension to phospholipid biochemistry. Certainly the discovery that this powerful lipid chemical mediator was a glycerophospholipid was sufficient to

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reorient our thinking on the role of lipids in metabolic processes. The further fact that this unique compound, identified as 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, possessed not only a glyceryl ether backbone but also contained a short chain fatty acyl residue, i.e., an acetic acid moiety, was startling and certainly novel. Inasmuch as dogma in this field dictated that the "usual" naturally occurring glycerophospholipids should contain fatty acyl residues at least sixteen carbons in length, the proposal that platelet activating factor had an acetic acid residue would not have been received with acceptance so early if direct chemical synthesis of this factor had not been achieved. To date, it is remarkable that the most potent platelet activating factor is that found as the predominant natural form, alkylacetylglyceryl ether phosphocholine. In fact, there is only a small window of acyl chain lengths, e.g., C2 to C4, in which biological activity is elicited. Furthermore, removal of the acetate group renders the resulting derivative, the lyso form, completely inactive. Research in this field has expanded at a dramatic pace and certainly one prime objective will be to learn how this molecule can cause such diverse metabolic effects at levels where perhaps 100 to 300 molecules (or less) interact with a cell. Future studies in this area of phospholipid biochemistry are most challenging as well as exciting.

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