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Phosphatidylserine Affects Specificity of Protein Kinase C Substrate Phosphorylation and Autophosphorylation†

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ABSTRACT: Protein kinase C substrate phosphorylation and autophosphorylation are differentially modulated by the phosphatidylserine concentration in model membranes. Both substrate phosphorylation and autophosphorylation display a cooperative dependence on phosphatidylserine in sonicated vesicles composed of diacylglycerol and either phosphatidylcholine or a mixture of cell lipids (cholesterol, sphingomyelin, phosphatidylethanolamine, and phosphatidylcholine). However, the concentration of phosphatidylserine required to support phosphorylation varies with individual substrates. In general, autophosphorylation is favored at intermediate phosphatidylserine concentrations, while substrate phosphorylation dominates at high phosphatidylserine concentrations. These different phosphatidylserine dependencies may reflect different affinities of particular substrates for negatively charged membranes. Increasing the negative surface charge of sonicated vesicles increases the rate of substrate phosphorylation. In contrast to the modulation exerted by phosphatidylserine, diacylglycerol activates protein kinase C equally toward substrate phosphorylation and autophosphorylation. These results indicate that both diacylglycerol and phosphatidylserine regulate protein kinase C activity in the membrane: diacylglycerol turns the enzyme on, while phosphatidylserine affects the specificity toward different substrates.

The Ca^{2+}/lipid-dependent protein kinase C plays a critical role in the transduction of extracellular signals that promote phospholipid turnover (Nishizuka, 1986, 1988). Receptor-mediated hydrolysis of phosphatidylinositol bisphosphate generates two important second messengers in the activation of protein kinase C: diacylglycerol and Ca^{2+}. Recent evidence suggests that one of these messengers, diacylglycerol, may also be formed by receptor-mediated hydrolysis of phosphatidylcholine (Besterman et al., 1986; Slivka et al., 1988). Protein kinase C becomes tightly associated with the plasma membrane in response to increased intracellular Ca^{2+} levels and is activated by diacylglycerol. The "translocation" of protein kinase C to the membrane in response to a variety of biological agonists, in many different cell types, is well documented (Kraft et al., 1982; Farrar & Anderson, 1985; Terbush & Holz, 1986). Nonetheless, the mechanism of membrane binding and activation of protein kinase C remains to be elucidated.

Protein kinase C binds to acidic membranes in a Ca^{2+}-dependent manner (Bazzi & Nelsestuen, 1987a). While this binding can usually be reversed by removal of Ca^{2+}, recent evidence indicates that, under the appropriate conditions, the binding may be irreversible (Bazzi & Nelsestuen, 1988b). Examination of binding to phospholipid monolayers and bilayers indicates that protein kinase C inserts into the hydrophobic core of the membrane (Bazzi & Nelsestuen, 1988a,b).

Activity of protein kinase C is dependent on phosphatidylserine (PS),[1] a lipid located on the cytoplasmic surface of the plasma membrane, and diacylglycerol (Kaibuchi et al., 1981; Boni & Rando, 1985; Bazzi & Nelsestuen, 1987a). The stoichiometry and specificity of the activation of protein kinase C by lipid has been determined by using a mixed micelle assay developed by Bell and co-workers (Hannun et al., 1985).

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[1] Abbreviations: DG, dioleoylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SM, sphingomyelin.

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Systematic variation of the number and species of lipid interacting with one monomer of protein kinase C has revealed that one molecule of diacylglycerol, or one molecule of phorbol ester, per protein kinase C monomer is sufficient to activate the enzyme (Hannun et al., 1985; Hannun & Bell, 1986). In contrast, ≥12 molecules of PS per protein kinase C monomer are required for full enzymatic activity (Newton & Koshland, 1989). The activation by phospholipid is highly cooperative and specific for the serine head group (Hannun et al., 1986; Newton & Koshland, 1989; Lee & Bell, 1989).

In this report we extend our findings on the cooperative activation of protein kinase C by micelle-bound PS to examine whether membrane-inserted PS is a sensitive regulator of protein kinase C function. We report that activation of protein kinase C by bilayer PS is cooperative and specific. Furthermore, the dependence on PS concentration for protein kinase C activity varies for autophosphorylation and for phosphorylation of different substrates. Thus, PS modulates the specificity and degree of protein kinase C activity.

Materials and Methods

Materials. Bovine brain L-α-phosphatidylserine (PS), egg L-α-phosphatidylcholine (PC), egg L-α-phosphatidylethanolamine (PE), and L-α-dioleoylglycerol (DG) were purchased from Avanti Polar lipids. Egg sphingomyelin (SM), cholesterol, histone H1, phenyl-Sepharose CL-4B, and polylysine–agarose were supplied by Sigma Chemical Co. DEAE-cellulose was from Whatman, AcA_{34} Ultrogel from LKB, and hydroxylapatite (Bio-Gel HT) from Bio-Rad. [γ-^{32}P]ATP was from Du Pont-New England Nuclear. All other chemicals were reagent grade. Protein kinase C was purified from a cytosolic extract of homogenized rat brain (Sprague-Dawley) by sequential DEAE, phenyl-Sepharose, gel filtration (AcA_{34}), polylysine–agarose (Huang et al., 1986a), and Mono Q (Jeng et al., 1986) chromatography. Mono Q chromatography resolved the protein kinase C into two peaks, the first of which was purified further by hydroxylapatite chromatography using a prepacked column supplied by Mitsui Toatsu Tokyo (Huang et al., 1986b). The hydroxylapatite-purified protein kinase C was free of Type III [nomenclature of Huang et al. (1986b)] as judged by staining with monoclonal antibodies to this isozyme. Unless otherwise noted, this protein kinase C was used in experiments (40 μg mL^{-1} protein; 45 units mL^{-1}). In some experiments partially purified protein kinase C [DEAE chromatography, ammonium sulfate precipitation, and AcA34 chromatography (Mochly-Rosen & Koshland, 1987)] was employed in order to examine phosphorylation of endogenous rat brain substrates of the enzyme; the concentration of protein in this preparation was approximately 0.3 mg mL^{-1}, and it had an enzymatic activity of 10 units mL^{-1}. Protein kinase C was stored at −20 °C in buffer containing 50% glycerol, 0.5 mM DTT, 0.5 mM EDTA, 0.5 mM EGTA, and 10 mM Tris, pH 7.4. Hydroxylapatite-purified samples contained 0–100 mM potassium phosphate. τ protein was a generous gift from R. D. Cole and J. Baudier.

Lipids. Small unilamellar vesicles of compositions noted in the figure legends were prepared by combining chloroform solutions of lipids, drying under N_2, and hydrating in buffer containing 20 mM Tris, pH 7.4. Suspensions (5 mM lipid) were sonicated to clarity in a bath sonicator (Branson, approximately 50 °C). Mixed micelles containing 2.5 mol % DG and 10 mol % brain PS in 0.5% (w/v) Triton X-100 were prepared by a modification (Newton & Koshland, 1989) of the procedure of Hannun et al. (1985).

Protein Kinase C Assay. The initial rates of [^{32}P]phosphate incorporation from [γ-^{32}P]ATP into saturating amounts of histone, τ, endogenous rat brain substrates, or protein kinase C were measured. *Histone*: Protein kinase C (50 μL of a 50-fold dilution of stock enzyme into 2 mM DTT and 20 mM Tris, pH 7.4), vesicles (8 μL of 5 mM lipid), and $CaCl_2$ (5 μL of 20 mM solution) were combined. The reaction was initiated by addition of 16 μL of solution containing histone H1 (100 μM), [γ-^{32}P]ATP [100 μM; 0.14 Ci (mmol of ATP)$^{-1}$], 75 mM $MgCl_2$, and 20 mM Tris, pH 7.4, and the samples were incubated at 30 °C for 3 min. Thus, the incubation medium contained 20 μM histone H1, 0.5 mM lipid, 1.3 mM $CaCl_2$, 20 μM ATP, 15 mM $MgCl_2$, 0.6% glycerol, 6 μM EDTA, 6 μM EGTA, 19 mM Tris, pH 7.4, and 1.3 mM DTT. The reaction was quenched by addition of 25 μL of a solution containing 0.1 M ATP and 0.1 M EDTA, pH 7, and aliquots (75 μL) were spotted on Whatman P81 ion-exchange paper and washed four times in 500 mL of 0.4% (v/v) phosphoric acid. τ: A 10-fold dilution of stock protein kinase C into buffer containing 20 mM Tris, pH 7.4, 2 mM DTT, and approximately 40 μg mL^{-1} τ was assayed as described above except that histone was omitted from the reaction mixture (concentration of τ in the reaction mixture was approximately 25 μg mL^{-1}). For some experiments, Triton X-100:PS:DG mixed micelles (85:10:5 mole ratio; 0.05% Triton X-100 in reaction mixture) replaced the vesicles. The reaction was terminated after incubation at 30 °C for 3 min by addition of 25 μL of SDS–PAGE sample buffer (0.265 M Tris, 8.4% SDS, 42% glycerol, 0.008% bromophenol blue and 20% β-mercaptoethanol, pH 6.8). Samples were heated at 100 °C for 3 min and analyzed by SDS–polyacrylamide gel electrophoresis [10% (Ames, 1974)] followed by autoradiography (Kodak X-Omat film). τ bands (45–66 kDa) were excised from dried gels and added to 10 mL of scintillation fluid (Scint A, Packard), and ^{32}P was detected by liquid scintillation counting. *Autophosphorylation*: The procedure for τ phosphorylation was followed except that ovalbumin (0.5 μg mL^{-1}) replaced τ in the dilution buffer. Samples were analyzed by autoradiography followed by excision and counting of the 80-kDa protein kinase C band. *67-kDa substrate*: Partially purified protein kinase C containing a 67-kDa protein as the major species was diluted 2-fold into 20 mM Tris, pH 7.4, 2 mM DTT, and 450 mM NaCl and assayed and analyzed as described for τ phosphorylation. The NaCl concentration was 150 mM in the reaction mixture.

Results

Histone Phosphorylation. The activation of protein kinase C toward histone phosphorylation displays a sigmoidal dependence on the PS content of sonicated vesicles. Figure 1 shows protein kinase C activity as a function of the PS concentration in sonicated vesicles containing 0–50 mol % PS, 45–95 % phosphatidylcholine, and 5 mol % DG. The protein:lipid ratio was on the order of 1:10^5, so that the membrane surface area was in large excess to that required for protein kinase C binding [approximately 100 lipids per protein kinase C, calculated from Bazzi and Nelsestuen (1987a)]. Increasing the protein kinase C concentration 2-fold did not alter the rate of histone phosphorylation catalyzed per protein kinase C, consistent with saturating concentrations of both substrate and vesicles (Figure 1, ●). Half-maximal activity was supported by vesicles containing a PS concentration of 10.2 mol %.

Figure 2 shows that sonicated vesicles containing a mixture of cell lipids, similar to that of the erythrocyte membrane, and diacylglycerol (2 mol %) activated protein kinase C with a sigmoidal dependence on the PS content of the membranes. Vesicles were composed of cholesterol, sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine (47:23:23:7

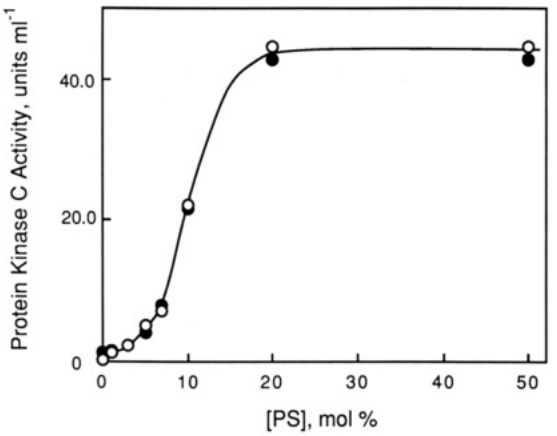

FIGURE 1: Dependence of histone phosphorylation on the PS content of sonicated vesicles. The rate of histone phosphorylation supported by sonicated vesicles (0.5 mM lipid) containing 0–50 mol % brain PS, 45–95 mol % egg PC, and 5 mol % DG was measured at two different protein kinase C concentrations: approximately 3 nM (○) and 6 nM (●) protein kinase C.

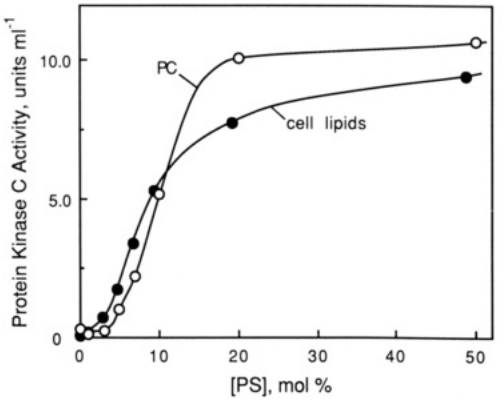

FIGURE 2: Effect of cell lipids on the PS dependence of histone phosphorylation. The rate of histone phosphorylation was measured in the presence of sonicated vesicles composed of lipids representative of the plasma membrane (cholesterol, sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine; 47:23:23:7 mole ratios) and diacylglycerol (2 mol %) (●) or phosphatidylcholine and diacylglycerol (5 mol %) (○) and 0–50 mol % PS. Lipid concentration: 0.5 mM.

mol ratio) and varying concentrations of PS. Compared to vesicles containing phosphatidylcholine as the bulk lipid, the amount of PS required for half-maximal activity decreased from 10.3 to 9.3 mol %. Similarly, a slight decrease in the steepness of the slope was observed, suggesting a decrease in the apparent cooperativity of the PS-dependent activation.

Autophosphorylation. The rate of autophosphorylation also displayed a sigmoidal dependence on the concentration of PS in sonicated PC/DG (5 mol %) vesicles (Figure 3); activity was half-maximal at 29 mol % PS. This is 2.5-fold higher than the concentration of PS supporting half-maximal histone phosphorylation.

τ Phosphorylation. The microtubule-associated protein τ, a substrate of protein kinase C (Baudier et al., 1986), requires high concentrations of PS in order to be phosphorylated. The PS dependence of τ phosphorylation is presented in Figure 4. The ratio of τ to protein kinase C was on the order of 20:1 (Figure 4A, lane 1). As for histone phosphorylation and autophosphorylation, a sigmoidal curve described the PS dependence of activity towards τ phosphorylation. In contrast to the rate of histone phosphorylation, which plateaued at 20 mol % PS in the bilayer, phosphorylation of τ by protein kinase C was insignificant at PS concentrations lower than 20 mol % (Figure 4A, lanes 2–6). Above 20 mol % PS, the rate of

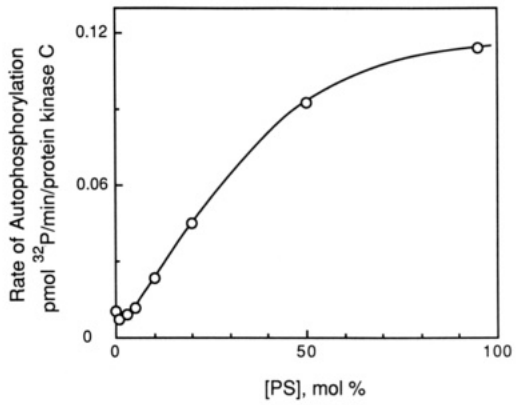

FIGURE 3: Dependence of autophosphorylation on the PS concentration in sonicated vesicles. The rate of autophosphorylation supported by vesicles composed of PC and DG (5 mol %) and 0–95 mol % PS was recorded as picomoles of phosphate incorporated per minute per total amount of protein kinase C in the assay (approximately 2.5 pmol). Lipid concentration: 0.5 mM.

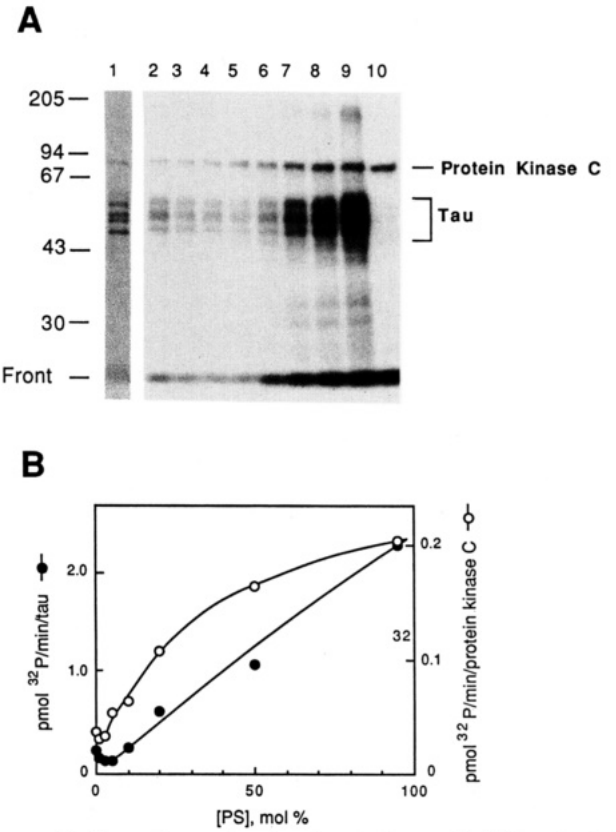

FIGURE 4: Dependence of τ phosphorylation on the PS content of sonicated vesicles. (A) τ phosphorylation was measured in the presence of vesicles containing 0–95 mol % PS, 5 mol % DG, and 0–95 mol % PC (0.5 mM lipid). Lanes 2–10 show an autoradiogram (4-h exposure, −70 °C): 0 mol % PS (lane 2), 1 mol % PS (lane 3), 3 mol % PS (lane 4), 5 mol % PS (lane 5), 10 mol % PS (lane 6), 20 mol % PS (lane 7), 50 mol % PS (lane 8), and 95 mol % PS (lane 9). The sample in lane 10 was incubated with Triton X-100 mixed micelles [10 mol % PS, 5 mol % DG, 0.05 % Triton X-100 (w/v)]. A Coomassie blue stain of a representative gel lane is shown in lane 1. (B) ^{32}P-Labeled bands from the gel in (A) were excised and analyzed by liquid scintillation counting. The rate of phosphorylation is expressed as picomoles of phosphate incorporated per minute per total amount of τ (●) or protein kinase C (○) present in the reaction mixture (approximately 2.5 pmol of protein kinase C per assay).

τ phosphorylation increased relative to the rate of autophosphorylation until PS was the only phospholipid in the bilayer (Figure 4, part A, lanes 7–9, and part B). Lane 10 shows phosphorylation resulting from incubation of protein kinase C and τ with Triton X-100:PS:DG mixed micelles

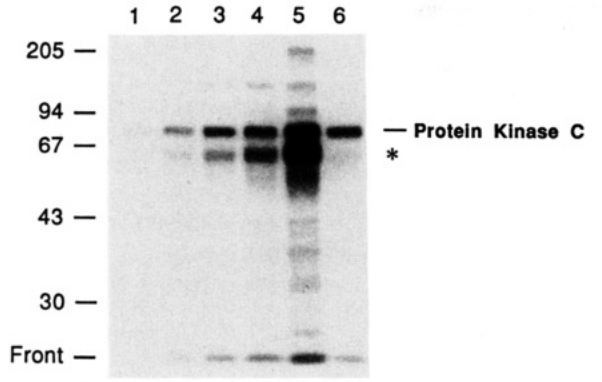

FIGURE 5: Phosphorylation of endogenous rat brain substrates present in partially purified protein kinase C preparation. Shown is an autoradiogram from a polyacrylamide gel of partially purified protein kinase phosphorylated in the presence of sonicated vesicles containing increasing concentrations of PS: 0 mol % PS (lane 1), 10 mol % PS (lane 2), 25 mol % PS (lane 3), 50 mol % PS, (lane 4), and 95 mol % PS (lane 5). Sonicated vesicles contained 5 mol % DG and PC as the remaining lipid; lipid concentration was 0.5 mM. Lane 6 shows the phosphorylation obtained in the presence of Triton X-100:PS:DG mixed micelles (85:10:5 mole ratios, 0.05% Triton X-100). An asterisk indicates the position of the major protein kinase C substrate. Molecular mass standards (in kDa) are shown on the left.

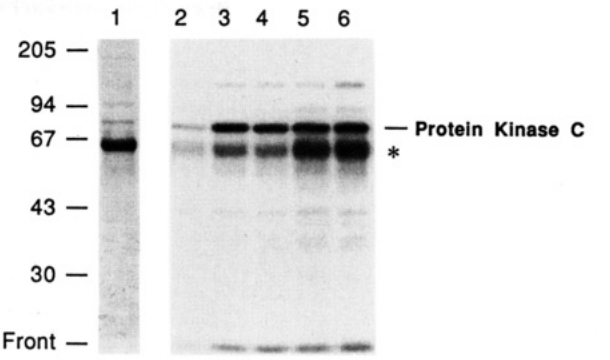

FIGURE 6: Effect of membrane surface charge on phosphorylation of 67-kDa endogenous rat brain substrate. Shown are a Coomassie blue stain (lane 1) and autoradiogram (lanes 2–6) of a gel containing partially purified protein kinase C incubated with sonicated vesicles containing varying amounts of PS, PA, and PC: 25 mol % PA (lane 2), 25 mol % PS (lane 3), equal concentrations of vesicles containing 25 mol % PA and vesicles containing 25 mol % PS (lane 4), 25 mol % PA and 25 mol % PS (lane 5), and 50 mol % PS (lane 6). Vesicles contained 5 mol % DG and 45–95 mol % PC; lipid concentration was 0.5 mM. An asterisk marks the position of the major protein kinase C substrate.

(85:10:5 mole ratio). While this composition of micelles supports maximum enzyme activity toward histone phosphorylation and autophosphorylation (Newton & Koshland, 1989), τ phosphorylation was not detectable. This may be because the ratio of τ to micelles was on the order of 1:10, so that the probability of τ binding to a micelle containing protein kinase C was $<10^{-3}$. Alternatively, the PS concentration in the micelles may not have provided sufficient surface charge to give effective τ binding. The rate of autophosphorylation was unaffected by the presence of τ in the assay mixture (compare Figures 3 and 4B). This is consistent with previous analysis, which indicated that the presence of substrate does not affect the PS dependence of autophosphorylation (Newton & Koshland, 1989).

Rat Brain Substrate Phosphorylation. Another substrate of protein kinase C regulated by PS is a rat brain protein copurifying with protein kinase C in the initial chromatography step (DEAE). This protein may be a member of the Ca^{2+}/lipid-binding family of proteins: it binds PS in a Ca^{2+}-dependent manner and comigrates with the Ca^{2+}/lipid-binding protein calelectrin (67 kDa) on SDS–polyacrylamide gels (not shown). Figure 5 shows that phosphorylation of this protein was insignificant at PS concentrations below 50 mol % PS (lanes 1–3) but was appreciable when PS was the only vesicle phospholipid (lane 5). As with τ phosphorylation, autophosphorylation dominated at low concentration of PS, while substrate phosphorylation dominated at high concentrations of PS. Also as was the case for τ, the 67-kDa substrate was not phosphorylated when the lipid was presented in the form of Triton X-100:PS:DG mixed micelles (85:10:5 mole ratio; lane 6).

Membrane Surface Charge. The possibility that membrane charge influences substrate phosphorylation was examined. This was suggested by the differential regulation of substrate phosphorylation by PS, indicating that this dependence was an intrinsic property of the substrate. Indeed, Bazzi and Nelsestuen (1987b) have shown that the ability to serve as a protein kinase C substrate is correlated with substrate binding to acidic membranes. The substrates examined in this report are all basic proteins that interact with PS-containing membranes (Nakadate et al., 1986; Bazzi & Nelsestuen, 1987b; and data not shown). Phosphatidic acid was chosen to modulate the surface charge of the vesicles, as this lipid is negatively charged yet does not contribute to the PS-dependent activation of protein kinase C (Newton & Koshland, 1989).

Figure 6 shows the phosphorylation of protein kinase C and 67-kDa substrate as a function of the surface charge of sonicated vesicles. Phosphatidic acid (25 mol %) in PC/DG vesicles produced no significant activation of protein kinase C (Figure 6, lane 2). A similar concentration of PS caused protein kinase C to autophosphorylate but did not stimulate significantly substrate phosphorylation (lane 3). When a population of vesicles containing PS (25 mol %) was mixed with vesicles containing phosphatidic acid (25 mol %), the resulting phosphorylation was the same as that obtained by vesicles containing PS (lane 4). (The total lipid concentration was held constant at 0.5 mM, so that the reaction mixture contained 0.25 mM vesicles with PS and 0.25 mM vesicles with PA. Under these conditions, 0.25 mM lipid provides a saturating concentration of vesicles.) If vesicles containing 25 mol % PS and 25 mol % PA (in the same bilayer) were incubated with protein kinase C, substrate phosphorylation increased 1.7-fold relative to autophosphorylation (lane 5). The degree of substrate phosphorylation stimulated by vesicles containing 25 mol % PA and 25 mol % PS was similar to that obtained by vesicles containing 50 mol % PS (lane 6). Thus, increasing the negative charge of the vesicle surface enhanced substrate phosphorylation relative to autophosphorylation.

Diacylglycerol. Figure 7 shows that, unlike phospholipid, diacylglycerol modulates substrate phosphorylation and autophosphorylation to the same degree. The rates of histone phosphorylation (●) and autophosphorylation (○) display a hyperbolic dependence on the diacylglycerol content of sonicated PC vesicles containing 20 mol % PS. This is consistent with the regulation of protein kinase C by diacylglycerol being an intrinsic property of the kinase rather than the substrate.

Discussion

The cellular regulation of protein kinase C function is a complex process involving structural changes in the protein and organizational changes in the plasma membrane. Receptor-mediated activation of the enzyme results in high-affinity binding of protein kinase C to the cytoplasmic surface of the plasma membrane, where its activity is regulated by membrane components. In order to gain a clearer under-

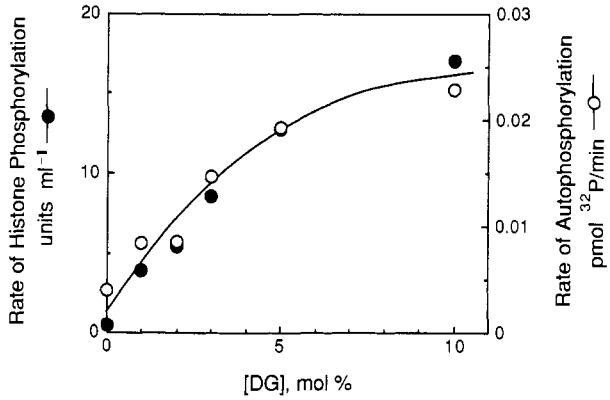

FIGURE 7: Dependence of protein kinase C activity on the diacylglycerol concentration of sonicated vesicles. The rates of histone phosphorylation (●) and autophosphorylation (○) were measured in the presence of vesicles containing 20 mol % PS, 70–80% PC, and 0–10 mol % DG. Lipid concentration was 0.5 mM. The rate of autophosphorylation is expressed as picomoles of phosphate incorporated per minute into the total amount of protein kinase C in assay (approximately 2.5 pmol of protein kinase C).

standing of how the membrane exerts its control on the enzyme, we have examined the regulation of enzymatic activity by lipids in model membranes. Because protein kinase C autophosphorylates by an intrapeptide reaction, this activity reflects directly the protein–lipid interaction and is a measure of intrinsic enzymatic activity (Newton & Koshland, 1989). This is in contrast to substrate phosphorylation, which reflects the interaction of at least three components: membrane, kinase, and substrate. We find that protein kinase C substrate phosphorylation and autophosphorylation are differentially regulated by PS and by membrane surface charge and that diacylglycerol regulates the intrinsic activity of the enzyme.

Cooperative Activation of Protein Kinase C by Phosphatidylserine. Phosphatidylserine in model membranes cooperatively activates protein kinase C. This is consistent with data obtained from a detergent:lipid mixed micelle assay, which revealed unusually high cooperativity ($n \geq 8$) in the activation of solubilized enzyme by PS (Hannun et al., 1985; Newton & Koshland, 1989). Using this mixed micelle assay, we showed that ≥12 molecules of PS are required to fully activate one monomer of protein kinase C. This led us to propose a model in which protein kinase C cooperatively sequesters at least 12 PS molecules around a membrane-interacting region of the protein, forming a domain enriched in this lipid (Newton & Koshland, 1989). The ability of bilayer PS to activate the enzyme cooperatively is consistent with such a model. Additionally, ongoing studies indicate that the binding of PS to protein kinase C is cooperative (Orr & Newton, 1990). The role of Ca^{2+} in phase partitioning PS around protein kinase C remains to be elucidated.

The activation of protein kinase C is remarkably specific for PS. Studies with monomeric protein kinase in the detergent–lipid mixed micelle assay indicated that only a few of the (≥12) PS molecules required to activate the detergent-solubilized enzyme can be replaced by lipids sharing some functional properties of the serine head group (negative charge, amino group). Consistent with this strict specificity, the cooperative activation by vesicle PS is relatively insensitive to changes in the bulk composition of the bilayer. PS cooperatively regulates protein kinase C activity in membranes composed of phosphatidylcholine or membranes containing lipids representative of the plasma membrane (cholesterol, sphingomyelin, phosphatidylethanolamine, and phosphatidylcholine). Only a slight decrease in cooperativity occurs in the latter membranes, perhaps because PE is contributing to the PS-stimulated activity. It is worth noting that the inclusion of cholesterol as half the membrane lipid did not significantly reduce the cooperative dependence of activity on PS content.

Membrane Surface Charge. Acidic phospholipids influence the ability of proteins to serve as substrates of protein kinase C. For many substrates (τ, 67-kDa endogenous rat brain substrate) the PS dependence of phosphorylation reflects a property of the substrate rather than the kinase, which is active at much lower PS concentrations. Increasing the membrane surface charge by addition of acidic phospholipids (PA) decreases the amount of PS required for substrate phosphorylation. Moreover, increasing the ionic strength of the medium decreases the ability of the substrate to be phosphorylated by protein kinase C (data not shown). This suggests that electrostatic interactions between the substrate and membrane are important in presenting the substrate to protein kinase C. These data are consistent with results of Bazzi and Nelsestuen (1987b), who correlated substrate phosphorylation with binding of substrate to acidic membranes. Thus, the membrane may provide a matrix that brings together substrate and kinase: the more negative the surface, the greater the binding of substrate (presumably through basic domains on the protein) and the more accessible to phosphorylation by protein kinase C. It is interesting to note that phosphorylation of substrates by the catalytic domain of protein kinase C, which is no longer regulated by PS, is also promoted by anionic phospholipids (Nakadate et al., 1987). Electrostatic interactions between the catalytic domain and the membrane may also play a role in regulating protein kinase C function.

Diacylglycerol. Diacylglycerol regulates the intrinsic activity of protein kinase C: substrate phosphorylation and autophosphorylation are influenced in the same way by this lipid. One molecule of diacylglycerol per protein kinase C molecule is sufficient to activate the kinase (Hannun et al., 1985). Thus, generation of this lipid in the plasma membrane may turn on protein kinase C, whose activity is then modulated by the phospholipid environment.

CONCLUSION

The foregoing results suggest that PS could modulate protein kinase C activity in vivo. In pure lipid membranes, protein kinase C intrinsic activity (measured by autophosphorylation) is sensitive to PS in the range of 5–50 mol % PS. The concentration of PS in the inner leaflet of most cells is on the order of 15 mol % of the total lipid (Verkleij et al., 1973), in a range affording sensitive control of enzyme function. Thus, protein kinase C intrinsic activity may be sensitive to the local concentration of PS in the plasma membrane. The existence of domains enriched or depleted in PS is not unreasonable, since a growing number of proteins have a high affinity for PS (Benfenati et al., 1989; Cohen et al., 1988; Bach et al., 1986). Such proteins may mask accessible PS, forming a local domain depleted in this lipid, or, if enough lipid is sequestered, may provide a domain enriched in PS. Furthermore, recent evidence suggests that PS metabolism is altered during signal transduction. PS synthesis increases during interleukin-1 signaling, supporting the possibility that fluctuations in the intrabilayer concentration of PS could regulate protein kinase C activity (Didier et al., 1988). Additionally, phorbol esters have been shown to inhibit PS synthesis in HL60 cells (Kiss et al., 1987). Regulation of enzyme function by phospholipid provides complexity and fine-tuning to the transduction of extracellular signals.

Substrate phosphorylation by protein kinase C is regulated additionally by anionic phospholipids. This suggests that proteins having an affinity for acidic phospholipids provide

a favorable environment for protein kinase C and are preferred substrates. Indeed, several recently identified protein kinase C substrates interact with acidic lipids: the glucose transporter of human erythrocytes is stimulated by PS and PA (Tefft et al., 1986) and its in vivo phosphorylation by protein kinase C has been reported (Witters et al., 1985), the insulin receptor is activated by the acidic lipid phosphatidylinositol (Sweet et al., 1987) and is a substrate of protein kinase C (Bollag et al., 1986), and a chromaffin granule-binding protein (chromobindin 9) interacts with PS and serves as a substrate of protein kinase C (Summers & Creutz, 1985). Thus, the surrounding lipid of proteins may serve as a matrix that attracts protein kinase C to a particular substrate.

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