Regulation of Platelet Protein Kinase C by Oleic Acid

The regulation of protein kinase C by oleic acid was studied, and parameters that characterize the activation of protein kinase C by oleic acid and distinguish its effects from those of diacylglycerol (DAG) and phosphatidylserine (PS) were delineated. Activation of protein kinase C by oleic acid required the presence of calcium and showed mild cooperative behavior (Hill number of 1.25) suggesting that Ca(oleate)2 is the active species. Kinetic analysis of the interaction of sodium oleate with substrates indicated that sodium oleate acted to increase the activity of the enzyme without modulating the $K_M$ for either MgATP or histone substrates. In this respect, sodium oleate action resembled that of DAG but not PS. However, multiple parameters distinguished the effects of sodium oleate from those of DAG. Unlike DAG, sodium oleate was unable to inhibit phorbol dibutyrate binding to protein kinase C. Sodium oleate also failed to interact with micelle-bound protein kinase C and preferentially activated "soluble" protein kinase C. The addition of histone caused protein/lipid aggregation in the presence of DAG but not in the presence of oleate. Activation of protein kinase C by sodium oleate or by PS/DAG demonstrated differential susceptibility to the action of inhibitors. Sphingosine and NaCl were more potent in inhibiting activation of protein kinase C by PS/DAG than by sodium oleate. Sodium oleate also expressed PS-like activity in that calcium and oleate acted as cofactors in activation of protein kinase C by DAG. Similar to PS, the ability of oleate to act in synergy with DAG resulted from "competitive" activation with a decrease in $K_M$(app) of protein kinase C for DAG. Finally, sodium oleate was unable to induce autophosphorylation of protein kinase C. These studies demonstrate that oleate activates protein kinase C by a mechanism that is distinct from PS/DAG but partially overlaps the kinetic effects of both PS and DAG. The significance of these studies is discussed in relation to mechanisms of protein kinase C activation and to the possible physiological relevance of activation of protein kinase C by fatty acids.

Protein kinase C, a calcium/phospholipid/diacylglycerol-dependent protein kinase, is a key element in cell regulation and signal transduction (1). The enzyme is physiologically activated in response to diacylglycerol (DAG) generated from membrane phospholipids (2). The enzyme is also activated by phorbol esters (3) and other tumor promoters, and it appears to be the predominant intracellular receptor for phorbol esters (4). Cloning of cDNA for protein kinase C from different tissues and different species resulted in the identification of a family of closely related isoenzymes that share similar structural and functional features (5). The enzyme is primarily organized into a carboxyl-terminal 50-kDa catalytic domain that interacts with ATP and protein substrate and an amino-terminal 32-kDa regulatory domain that transduces the effects of calcium, lipid modulators, and phorbol esters (6-8).

In vitro, protein kinase C is also activated by cis-unsaturated fatty acids (9, 10). The mechanism by which fatty acids modulate the activity of protein kinase C remains, however, poorly understood. Fatty acid activation of protein kinase C is independent of phosphatidylycerine (PS) (9). Studies on the interaction of fatty acids with calcium and DAG have yielded conflicting results. Oleic acid activation of protein kinase C has been shown to be independent of calcium in some studies (9) and to be totally dependent on calcium in others (11-13) with the $\gamma$ isofrom of protein kinase C showing independence from calcium (12) and $\alpha$ and $\beta$ isoforms being dependent on calcium (12). DAG has been shown to be without effect on fatty acid activation on protein kinase C (12), to slightly modulate the effects of fatty acids (11), or to strongly synergize with fatty acids (14).

Elucidation of the mechanisms of interaction of fatty acids with protein kinase C is of 2-fold significance. First, it allows insight into the mechanism of activation of protein kinase C. Second, such studies are essential for evaluating possible physiologic regulation of protein kinase C by arachidonic and other unsaturated fatty acids. In this report, we examine the in vitro regulation of platelet protein kinase C by oleic acid. We elucidate important parameters that characterize the interaction of oleic acid with protein kinase C and distinguish the effects of oleic acid from those of PS or DAG. These include kinetic parameters, effects on phorbol binding, effects on autophosphorylation, effects on substrate-induced aggregation, and susceptibility to inhibitors. These studies provide new information on proposed models for protein kinase C activation and on possible physiologic interaction of fatty acids with protein kinase C.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**Kinetic Analysis of Allosteric Regulation of Protein Kinase C by Sodium Oleate—In vitro, protein kinase C is activated**

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*The abbreviations used are: DAG, diacylglycerol; PS, phosphatidylycerine; DiC1,1, sn-1,2-dioleoylglycerol.

† Portions of this paper (including "Experimental Procedures," part of "Results," and Figs. 1 and 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
in the presence of calcium, PS, and DAG. The kinetic interactions between lipid modulators, calcium, MgATP, and histone substrate were previously studied using mixed micellar methodology (18, 19, 24). These studies showed that the modulation of protein kinase C activity by PS and DAG could be kinetically distinguished. Both PS and DAG activated the enzyme without changing the apparent affinity of the enzyme for MgATP substrate (20). On the other hand, PS acted as a "competitive activator" of protein kinase C with respect to histone substrate, whereby PS decreased the apparent KM for histone without modifying the Vmax. DAG, however, did not modulate the KM for histone substrate but acted to increase the Vmax and kcat. (20). These results were interpreted to indicate that the effects of PS are consistent with a "K-system" whereby, in a first step, the interaction of PS with protein kinase C changes the affinity of the enzyme to substrates. This is consistent with the "pseudosubstrate" hypothesis, whereby activation of the enzyme results in release of the auto-inhibitory pseudosubstrate from the protein substrate site (25). However, this is not sufficient for activation of the enzyme since catalytic activity remains low (20). In the second stage of activation, protein kinase C is specifically activated by the addition of either DAG or phorbol ester. The effects of DAG were interpreted to be consistent with a "V-system," whereby DAG activates the enzyme by increasing its catalytic rate (20).

With these considerations in mind, we examined the effects of sodium oleate on protein kinase C and studied its interaction with MgATP and histone substrates. The dependence of protein kinase C on MgATP was studied at different fixed concentrations of sodium oleate in the presence of 400 μM calcium and saturating concentrations of histone, and in the absence of other lipid cofactors. Double-reciprocal plots of activity versus MgATP concentrations at increasing concentrations of sodium oleate resulted in a family of linear plots intersecting at the x axis (Fig. 3); that is, sodium oleate modulated the Vmax but not the KM of protein kinase C for MgATP. These results suggest that sodium oleate does not modulate the affinity of the enzyme for MgATP substrate. These results are similar to the effects of PS and DAG on the MgATP dependence of protein kinase C (20).

An important kinetic parameter differentiating the effects of PS from those of DAG is the modulation of the KM for histone by PS but not by DAG (20). Therefore, the effects of sodium oleate on the histone dependence of protein kinase C was evaluated. Double-reciprocal plots of activity versus histone concentration, at variable concentrations of sodium oleate, resulted in linear plots (Fig. 4). These plots intersected at the x axis showing that sodium oleate does not modulate the KM of protein kinase C for histone. Therefore, with regards to this parameter, sodium oleate appears to activate the enzyme by a mechanism similar to that of DAG and not PS; i.e. by increasing the Vmax and kcat for histone but not decreasing the KM.

These studies show that, in activating protein kinase C, sodium oleate behaves kinetically similar to DAG but not PS. However, unlike DAG, sodium oleate does not require the presence of phospholipid cofactor for enzyme activation (9).

**Effects of Sodium Oleate on Phorbol Binding—**Protein kinase C serves as the major intracellular receptor for phorbol esters (4). Phorbol dibutyrate binding to protein kinase C requires calcium and phospholipid as cofactors, and is competitively inhibited by DAG (24). This, in addition to structural similarities between phorbol esters and DAGs, has been taken to strongly suggest that DAGs and phorbol esters interact at a common site (2).

If the kinetic similarity in activation of protein kinase C by sodium oleate and DAG is a result of interaction at a common site, then sodium oleate should be able to inhibit phorbol dibutyrate binding. Therefore, the effects of sodium oleate on phorbol dibutyrate binding to protein kinase C were investigated. Over a concentration range of 10–200 μM, sodium oleate showed little effect on specific binding of phorbol dibutyrate to protein kinase C (Fig. 5). Over the same concentration range, sodium oleate activated platelet protein kinase C up to 70% of maximal activation achieved with saturating concentrations of PS and DAG (not shown). In contrast, Dic3-S1 showed potent inhibition of phorbol dibutyrate binding to protein kinase C under similar conditions (24). Therefore, while sodium oleate is able to significantly activate protein kinase C by a kinetic mechanism resembling that of DAG, the site of action of sodium oleate must be distinct from that of phorbol ester/DAG since it is unable to compete off specific phorbol binding.

![Fig. 3. Effects of sodium oleate on the MgATP dependence of protein kinase C. Double-reciprocal plots of activity and MgATP concentration constructed at several fixed concentrations of sodium oleate (NaO) as indicated.](https://example.com/fig3)

![Fig. 4. Effects of sodium oleate on the histone dependence of protein kinase C. Double-reciprocal plots of activity and histone concentration constructed at the indicated concentrations of sodium oleate (NaO).](https://example.com/fig4)

![Fig. 5. Effects of sodium oleate on phorbol binding. Lack of inhibition of phorbol dibutyrate binding to protein kinase C by sodium oleate. ](https://example.com/fig5)
Protein Kinase C Regulation by Oleic Acid

Differential Effects of Sodium Oleate on Soluble Versus Micelle-bound Protein Kinase C—The low micromolar concentrations of sodium oleate at which it activates protein kinase C are well below the critical micellar concentration of sodium oleate\(^3\) (9). This has led to the suggestion that sodium oleate interacts with soluble enzyme (9). To investigate whether sodium oleate preferentially interacts with "soluble" protein kinase C or whether it is also able to activate membrane-bound enzyme, the ability of sodium oleate to activate micelle-bound protein kinase C was investigated. Previous studies showed that protein kinase C can tightly bind to Triton X-100/PS mixed micelles in the presence of calcium. Under these conditions, the enzyme shows little activation by PS and calcium, but all enzyme molecules monomerically bind to mixed micelles (18). Therefore, protein kinase C was allowed to interact with Triton X-100/PS mixed micelles in the presence of calcium under conditions where it strongly binds to those micelles. The addition of sodium oleate to micelle-bound protein kinase C failed to modulate enzyme activity (Fig. 6). Over the same concentration range, sodium oleate strongly activated soluble protein kinase C in the absence of detergents or lipids (Fig. 6). These studies support the suggestion that sodium oleate activates soluble protein kinase C and further indicate that sodium oleate is unable to interact with surface-bound enzyme, possibly due to a common site of interaction of PS and oleate with protein kinase C.

Effects of Sodium Oleate on Substrate-induced Aggregation—Previous studies have shown that addition of histone substrate to protein kinase C in the presence of lipid vesicles or detergent/lipid mixed micelles results in changes in light scattering (21). This has been interpreted to indicate the formation of aggregates of lipids and protein. Since sodium oleate activates soluble protein kinase C at low concentrations, the effects of sodium oleate on light scattering were studied. Light scattering was measured under conditions identical to those for protein kinase C assay; that is in the presence of Ca\(^{2+}\), MgATP, Mg\(^{2+}\), Tris-HCl, protein kinase C, and the indicated lipid cofactors. Under these conditions, the addition of histone substrate to protein kinase C in the presence of PS/DAG vesicles resulted in an increase in light scattering (Fig. 7). A reversible increase was noted when histone was added in the presence of Triton X-100/PS/DAG mixed micelles but not in the presence of Triton X-100/PS mixed micelles (Fig. 7). However, when histone was added to protein kinase C in the presence of sodium oleate, no increases in light scattering were observed (Fig. 7). These studies show that sodium oleate has unique effects on lipid-protein and protein-protein interactions during activation of protein kinase C. They also strongly support the hypothesis that sodium oleate causes activation of soluble protein kinase C without prior interaction with membranes, surfaces, or aggregates of substrates.

Differential Effects of Inhibitors on Sodium Oleate-induced Activation of Protein Kinase C—Activation of protein kinase C by PS/DAG is subject to different types of inhibition resulting from the action of inhibitors at the regulatory domain (e.g. sphingosine) (26, 27), at the MgATP site (e.g. H7) (28), and possibly at the substrate site (e.g. ionic strength) (20). Therefore, examining the differential effects of inhibitors on activation of protein kinase C by sodium oleate or by PS/DAG could yield important information on mechanisms of activation.

Sphingosine is a potent and reversible inhibitor of protein kinase C by DAG/phorbol esters. It is also a potent inhibitor of phorbol ester binding to protein kinase C (26). These effects appear to be secondary to inhibition of the interaction of DAG/phorbol esters with the membrane-bound enzyme. To evaluate the effects of sphingosine on activation of protein kinase C by sodium oleate and to compare those effects with activation by PS/DAG, protein kinase C activity was measured using lipid vesicles since Triton X-100 inhibits the effects of sodium oleate, thus precluding the use of mixed micelles. Protein kinase C was, therefore, activated in the presence of 25 \(\mu\)M PS/DAG (20 \(\mu\)M PS and 5 \(\mu\)M DiC\(_{18:1}\)) or 25 \(\mu\)M sodium oleate. Sphingosine produced a dose-dependent inhibition of activation of PS/DAG (Fig. 8) with inhibition seen at concentrations of sphingosine as low as 3 \(\mu\)M. Activation of protein kinase C, however, by sodium oleate was more resistant to sphingosine action with no inhibition seen with up to 10 \(\mu\)M sphingosine (Fig. 8). Significant inhibition was seen at concentrations of sphingosine greater than 25 \(\mu\)M. Since sphingosine preferentially interacts with the lipid regulatory domain of protein kinase C, these studies suggest that sodium oleate activates protein kinase C by a mechanism distinct from that of either PS or DAG. As an additional implication, these studies show that sphingosine may not be a potent inhibitor of protein kinase C activated by fatty acids in vivo.

In contrast to sphingosine, the isoquinolinesulfonamide H7 inhibits protein kinase C activity by competing with MgATP. H7 was equipotent in inhibiting protein kinase C activation by PS/DAG or by sodium oleate (Fig. 9) with half-maximal inhibition occurring at a concentration of H7 of approximately 25 \(\mu\)M.

Activation of protein kinase C by PS/DAG is also subject

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\(^3\)The solubility of oleate at pH 7.5, however, is significantly modified by the presence of oleic acid. Further studies are required to determine the physical structure of oleate/oleic acid at pH 7.5 in the low micromolar range (38).
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Sphingosine (PM)

FIG. 8. Effects of sphingosine on protein kinase C activity. Protein kinase C was activated with either PS/DAG vesicles containing 20 \( \mu \text{M} \) PS and 5 \( \mu \text{M} \) \( \text{DiC}_{18:1} \) or with sodium oleate (25 \( \mu \text{M} \)). The effects of increasing concentrations of sphingosine on protein kinase C activity were then evaluated. Results are expressed as percent of activity without inhibitor.

FIG. 9. Effects of H7 on protein kinase C. Protein kinase C was activated in the presence of sodium oleate (25 \( \mu \text{M} \)) or in the presence of PS/DAG vesicles containing 20 \( \mu \text{M} \) PS and 5 \( \mu \text{M} \) \( \text{DiC}_{18:1} \). The effects of increasing concentrations of H7 on protein kinase C activity were then determined.

FIG. 10. Inhibition of protein kinase C activity by increasing salt concentration. Protein kinase C was activated with either sodium oleate (25 \( \mu \text{M} \)) or PS/DAG vesicles containing 20 \( \mu \text{M} \) PS and 5 \( \mu \text{M} \) \( \text{DiC}_{18:1} \). The effects of increasing NaCl concentration on protein kinase C activity were then determined. Results are expressed as percent of activity without inhibitor.

Synergy in Protein Kinase C Activation by Sodium Oleate and DAG—It became clear from the above studies that sodium oleate does not interact with protein kinase C at the phorbol ester/DAG site. Although sodium oleate shared similar kinetic parameters with DAG, there were important differences between the two. The most notable difference is that activation of protein kinase C by DAG requires prior interaction of enzyme with PS/calcium (18). This initial interaction results in membrane-association of protein kinase C and an increase in the affinity of the enzyme for protein substrate. Since, at concentrations that activate protein kinase C, sodium oleate did not modulate the affinity of the enzyme for histone substrate, a "PS-like" role for sodium oleate was suspected; i.e. at presumably lower concentrations, sodium oleate is able to modulate the affinity of the enzyme to protein substrate without activating the enzyme. Kinetic elucidation of such a role for sodium oleate would be masked by the predominant effect of sodium oleate in increasing \( k_m \). However, a corollary to such a role implies that sodium oleate may function as a cofactor for DAG activation of protein kinase C. Therefore, the interaction between sodium oleate and DAG was studied. \( \text{DiC}_{18:1} \) was unable to activate protein kinase C in the absence of phospholipid cofactor (Fig. 11). The addition of sodium oleate resulted in synergistic activation of protein kinase C (Fig. 11A). Thus, while sodium oleate activated protein kinase C, it was also able to act as a cofactor for DAG allowing DAG to activate protein kinase C in the absence of phospholipid cofactors (Fig. 11B). The ability of oleate to replace PS supports a PS-like role for oleate. Also, the inability of oleate to interact with the protein kinase C-PS-Ca\(^{2+}\) complex (Fig. 6) suggests that PS and oleate interact at a common site.

Effects of Sodium Oleate on Autophosphorylation of Protein Kinase C—The above studies suggest that sodium oleate activates protein kinase C by a novel mechanism distinct from that of PS/DAG. If this is so, then sodium oleate may have protein substrate that is more resistant to inhibition by salts.

Effects of Sodium Oleate on Autophosphorylation of Protein Kinase C—The above studies suggest that sodium oleate activates protein kinase C by a novel mechanism distinct from that of PS/DAG. If this is so, then sodium oleate may have
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Also failed to cause aggregation of enzyme and protein substrate. Finally, activation of protein kinase C by sodium oleate was more resistant to ionic strength inhibition. These results strongly show that sodium oleate activates protein kinase C in vitro by a mechanism distinct from that of PS/DAG.

These studies have important implications concerning the in vitro mechanism of regulation of protein kinase C. Mechanistically, sodium oleate preferentially activates soluble rather than membrane-bound protein kinase C. The action of sodium oleate occurs at a site distinct from that of DAG/phorbol esters. At this point, it cannot be determined whether sodium oleate also interacts at the PS site or not, although the inability of oleate to interact with protein kinase C in the presence of PS suggests a common site for the interaction of PS and oleate with protein kinase C. Two lines of evidence suggest that sodium oleate may have two sites of interaction with protein kinase C. First, activation of protein kinase C by sodium oleate shows positive cooperativity with the requirement for at least two molecules of sodium oleate. Second, the synergy studies between sodium oleate and DAG suggest that sodium oleate may have two functions; sodium oleate partially mimics the requirement for PS, and sodium oleate can directly replace the ability of DAG to increase the $k_{cat}$ of the enzyme. Further elucidation of the mechanism of regulation of protein kinase C by sodium oleate would require determination of the domains of protein kinase C responsive to sodium oleate using mutational analysis of protein kinase C.

Another implication of these studies concerns the pharmacologic and physiologic regulation of protein kinase C by activators and inhibitors. Unlike arachidonic acid, the metabolism of sodium oleate does not lead to the formation of bioactive metabolites. Therefore, sodium oleate may be used as a probe to explore protein kinase C activation. Such studies have already shown the feasibility of the regulation of protein kinase C in cell systems by sodium oleate and other fatty acids (30-34), although it has not been established whether this is a result of direct effects or not.

If protein kinase C is physiologically activated by unsaturated fatty acids, the above studies suggest that inhibitors acting at the lipid-binding domain, such as sphingosine, may not be potent or useful inhibitors in this situation.

A major question regarding the activation of protein kinase C by unsaturated fatty acids is whether these molecules have a role in physiologically activating protein kinase C bypassing the requirement for DAG. The above studies show that sodium oleate preferentially activates soluble protein kinase C and is unable to interact with membrane-bound enzyme. Therefore, unsaturated fatty acids may have a physiologic role in activating soluble protein kinase C leading to phosphorylation of a distinct pool of substrates than those caused by phorbol esters/DAGs. Ongoing studies on intact and fractionated human platelets appear to support this hypothesis. Two additional features may have physiologic relevance. First, the in vitro studies suggest that sodium oleate acts in synergy with DAG in activating protein kinase C and this may suggest that, physiologically and pharmacologically, unsaturated fatty acids may potentiate protein kinase C activation by DAG. The inability of sodium oleate to interact with membrane-bound enzyme, however, may preclude such a physiologic role. Studies in human platelets show that pharmacologic application of unsaturated fatty acids and DAGs may synergize in platelet activation (35), although it has not been determined whether these are direct effects on protein kinase C.

\[ \text{\footnotesize{W. Khan, S. El Touny, and Y. Hannon, manuscript in preparation.}} \]
mechanistic studies on the nature of synergy between DAG and sodium oleate are required to elucidate this point. Second, sodium oleate is unable to cause autophosphorylation of protein kinase C. Although the precise significance of autophosphorylation of protein kinase C is not fully determined, autophosphorylation appears to modulate the ability of protein kinase C to interact with membranes (29, 36), and may enhance proteolysis and down-regulation of the enzyme (37). Therefore, activation of protein kinase C by unsaturated fatty acids may result in differential interactions of protein kinase C with membranes than enzyme activated by DAG, and may preclude down-regulation.

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EXPERIMENTAL PROCEDURES

Materials

Fresh frozen platelets were used as the source of protein kinase C (PKC) and were from Dupont New England Nuclear. Oleic acid, phosphatidic acid, Triton X-100 and histone type IBS were from Sigma. 1,2-di-oleoyl-sn-glycero-3-phosphoglycerol and 1,2-Di-oleoyl-sn-glycero-3-phosphocholine were from Avanti Polar Lipids, Inc. 1,2-Di-oleoylglycerol was prepared from dioleoylglycerol-3-phosphosphate as described (15).

Methods

Purification of Protein Kinase C --- Protein kinase C was purified from platelet cytosol to homogeneity as described (18) in a specific activity of 1.3 x 10^3 pmol/mg/min. Protein kinase C isoforms were separated by hydroxylapatite chromatography as described (17). The major cytosolic isoenzyme was protein kinase C δ (data not shown), and it was used for these studies.

Mixed Micellar Assay for Protein Kinase C --- Protein kinase C activity was assayed using Triton X-100 phosphatidylserine/DAG mixed micelles as previously described (18,19). The standard assay was performed in the presence of 0.5% Triton X-100 (v/v), 10 mM Tris pH 7.5, 20 mM DAG, 100 mM NaCl, 200 mM NaF, 10 mM MgCl₂, 10 mM ATP, and 20 mM of NaOH pH 7.5.

Vesicle Assay for Protein Kinase C --- Protein kinase C was also assayed with lipids delivered to vesicles rather than microinjected. The reaction assay was the same components as described above for the mixed micellar assay except that lipids were prepared in vesicle form. PS vesicles and PtdSer/DAG vesicles were prepared by drying down the lipids and resuspending in 1 ml of water. The vesicles were then sonicated with a probe sonicator on ice with 4 cycles of 30 sec each. Sodium oleate was added from an aqueous solution alone or in the presence of other lipids as indicated.

(PS) Fru6P Binding Assay for Protein Kinase C --- Phosphatidylserine binding to protein kinase C was performed as described (4) at 50 mM Fru6P and 50 μM PS. Specific binding was determined by subtracting non-specific binding (in the presence of 1 μM cold Fru6P) from total binding.

Protein Kinase C Autophosphorylation --- Protein kinase C was autophosphorylated in a standard reaction assay using either sodium oleate alone or PtdSer/DAG vesicles in the presence of 400 μM calcium. The reactions were conducted as previously described (20). Autophosphorylated PKC was separated on SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. The phosphorylated protein kinase C band was excised and counted in an LKB Beta scintillation counter in 10 ml of scintillation liquid.

Light Scattering Measurements --- Measurements of light scattering were used to qualitatively estimate protein-protein and protein-lipid interaction essentially as described by Inagami and Nishimura (21). Measurements were made on an Aminco Bowman spectrophotofluorometer. Excitation and emission wavelengths were 320 nm.

Preparation of Sodium Oleate --- Oleic acid was diluted in ethanol to form the desired stock solution, and then it was neutralized with sodium hydroxide to form the sodium salt.

Data Analysis --- Kinetic data were transformed according to the Lineweaver-Burk double reciprocal plot and Hill plot. Untransformed data were fitted by non-linear regression using EG CFT program as developed by Porrill (22). This procedure tests for best fit of data among different kinetic models and circumscribes inherent bias of Lineweaver-Burk analysis toward the lower range of cofactor substrate concentrations.

RESULTS

Activation of Protein Kinase C by Sodium Oleate --- Sodium oleate activated purified platelet protein kinase C in a dose-dependent manner (Fig. 1A). Maximal activation with sodium oleate was approximately 50% that achieved with saturating concentrations of PtdSer/DAG. Half maximal activation was reached at around 6 μM sodium oleate and full activation was achieved at 10 μM. A double reciprocal plot of activity vs sodium oleate was not linear but showed curvature (Fig. 1B). Hill plots showed a linear plot with a Hill number of 1.25 indicating positive cooperativity (Fig. 1). This suggests that protein kinase C interacts with at least two molecules of oleate. Cooperativity has also been observed for activation of protein kinase C by PtdSer with Hill numbers of 4-5 (18,19).

Figure 1. Activation of protein kinase C by sodium oleate. Hill plot of protein kinase C activity and concentration of sodium oleate.

Figure 2. Calcium dependence of activation of protein kinase C by sodium oleate. Protein kinase C activity was assayed in the presence of different concentrations of sodium oleate in the presence (0.4 μM calmodulin) or in the absence (10 mM EGTA) of calcium.
Regulation of platelet protein kinase C by oleic acid. Kinetic analysis of allosteric regulation and effects on autophosphorylation, phorbol ester binding, and susceptibility to inhibition.

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