Domain Structure and Phosphorylation of Protein Kinase C*

(Received for publication, March 21, 1986, and in revised form, July 21, 1986)

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The phospholipid- and calcium-dependent protein kinase C has been shown to autophosphorylate on both the catalytic and the regulatory domains. The auto-
phosphorylation displays zero-order kinetics, indicating that it is an intramolecular event. Autophosphorylation increases the activity of protein kinase C by
decreasing the $K_m$ for the substrate H1 histone. The catalytic fragment obtained by limited proteolysis can no longer autophosphorylate and has a reduced affinity for H1 histone, exhibiting a $K_m$ 5-fold higher than that of the intact enzyme. Monoclonal antibodies produced against the enzyme can distinguish between the cata-
lytic fragment and the intact enzyme by inhibiting their activities in a specific manner. Evidence suggesting that dimerization of protein kinase C occurs upon activation is presented.

Protein phosphorylation has been shown to be a major route for signal transduction in normal as well as transformed cells. Many protein kinases responsible for such activity have been described (1–3). Of particular interest is the calcium-
activated, phospholipid-dependent protein kinase C (4, 5). The activation of protein kinase C is coupled to phosphoryl-
diolinositol turnover and, specifically, to the appearance of diacylglycerol in the cell membrane (6). Small quantities of diacylglycerol sharply increase the apparent affinity of the enzyme for Ca$^{2+}$ and phosphatidylserine (7). Tumor-promoting
phorbol esters can substitute for diacylglycerol at very low
concentrations and activate protein kinase C in vivo (4), a common property of a
substrate (2). Protein kinase C has been reported to undergo autophosphorylation (4), a common property of a
number of protein kinases (11–13).

In this study, we have used a kinetic approach to charac-
terize autophosphorylation and its effect on kinase activity. Monoclonal antibodies were used to identify the kinase do-
main.

**EXPERIMENTAL PROCEDURES**

MATERIALS—The [γ-32P]ATP (20–40 Ci/mmol) used in this study
was from New England Nuclear. DEAE-cellulose, phenyl-Sepharose
CL-4B, Blue Sepharose, histone H1-S, L-α-phosphatidyl-l-serine, 1,2-
diolein, Triton X-100, and trypsin inhibitor from soybean were
from Sigma. ACA 34 Ultrogel was purchased from LKB (Vilnius,
La Garenne, France) and Scint A from Packard Instrument Co.
(Downers Grove, IL). All other chemicals used were reagent grade.

Protein Kinase C—30 male Sprague-Dawley rats (150–200-g) were
killed by cervical dislocation and their brains dissected out quickly at
4°C. Protein kinase C was purified by modification of the method of Kikkawa et al. (4). Briefly, the cytosol and 1% Triton-extracted
particulate fractions from the rat brain homogenates were subjected
to DEAE-cellulose chromatography, 66% ammonium sulfate precipi-
tation, gel filtration on ACA 34, Blue Sepharose, and phenyl-Sepha-
rose chromatography. The final enzyme preparation was in 20 mM
Tris-HCl, pH 7.4, containing 1 mM EGTA,1 1 mM EDTA, and 10%
glycerol (DEAE buffer) and exhibited a specific activity of 1000 units/
mg using H1 histone as substrate. The yield from the Blue Sepharose
was very poor and typically represented 10% of the amount of enzyme
obtained from the sizing column. Furthermore, this enzyme was found
to be less stable upon storage in 50% glycerol at −20°C. We therefore
used the enzyme obtained from the sizing column in most of the
experiments. Typically, this enzyme had a specific activity of 50–100
units/mg and was stable for more than a year at −20°C with 50%
glycerol. Its stimulation index, defined as the activity in the presence
of protein kinase C activators divided by the activity in the presence
of EGTA, was usually between 80 and 100.

Protein Kinase C Assay—Protein kinase C was routinely assayed
by measuring the incorporation of radioactive phosphate from [γ-
32P]ATP into H1 histone in the presence and absence of Ca$^{2+}$ (120
mM), diacylglycerol (0.4 μg), and phosphatidylserine (20 μg) in a
final volume of 55 μl as described by Kikkawa et al. (4). To 10–50 μl
of enzyme preparation, 16 μl of phospholipid mixture (prepared by
sonication) and Ca$^{2+}$ were added. The reaction was started by the
addition of 16 μl of ATP (1.25 mM, 2–4 X 10^6 cpm/mmol), MgCl2
(2.4 mM), H1 histone (100 μg) in 20 mM Tris-HCl, pH 7.4 (histone
phosphorylation mixture), and was allowed to proceed for 3 min at
30°C. The reaction was stopped by the addition of 25 μl of 0.2 M
EDTA and 0.2 M ATP. 50–80 μl of the mixture were spotted on 1.5-
cm² No. 3MM Whatman papers and washed sequentially for 5–10
min, in 500 ml of 10% ice-cold trichloroacetic acid, 500 ml of 5% ice-
cold trichloroacetic acid, 500 ml of 5% trichloroacetic acid at room
temperature, and 500 ml of 95% ethanol. The papers were placed in
siccillation vials with 5 ml of scintillation fluid and counted. The
background was always less than 0.1% of the total radioactivity.
(During this assay, the reaction proceeds linearly with time, and the
activity is proportional to the amount of the enzyme used.)

Autophosphorylation and Phosphorylation of Endogenous Sub-
strate—The reaction was done under the same conditions as for the
protein kinase C assay except H1 histone was not included (auto-
phosphorylation mixture). The reaction was stopped by adding PAGE
sample buffer (14) and heating to 80°C for 5 min. The amount of
P incorporated to protein was then measured by analysis on SDS-
PAGE (14). The quantitation was done by scanning the autoradi-
ograms on a densitometer (E. C. Apparatus Corporation) and mea-
suring the peak areas using a 3390A Hewlett-Packard integrator.
Alternatively, the areas desired were cut out of the gel and counted
in 5 ml of scintillation fluid.

The Effect of Autophosphorylation on the Activity of Protein Kinase C—In order to test the effect of autophosphorylation of protein kinase C
on its H1 histone phosphorylation activity, the following assay was done.
Protein kinase C was allowed to autophosphorylate under the
conditions described above, but in the absence of radio labeled ATP.

1 The abbreviations used are: EGTA, ethylenbis(oxyethyl-
enitrilo)tetraacetic acid, SDS-PAGE, sodium dodecyl sulfate-poly-
acrylamide gel electrophoresis.
After 6 min, H1 histone at concentration ranges of 0.05–50 μM and [γ-32P]ATP (2 × 10^6 cpm/nmol) were added to the reaction mixture. The reaction then proceeded for another 3 min at 30 °C, and the amount of 32P incorporated into histone was measured as in the standard assay. The K_m and V_max values were calculated on a double-reciprocal plot. K_m values changed slightly from one enzyme preparation to another. Moreover, a fraction of the kinase molecules were autophosphorylated during the 3-min assay, resulting in a heterogeneous population. The K_m values given were obtained from experiments done in triplicate using the same protein kinase C preparation. The decrease in K_m for the substrates seen after autophosphorylation was found in all enzyme preparations tested. However, the decrease in K_m varied from 1.5- to 4-fold for different protein kinase C preparations. Values computed from a representative experiment are given in the text.

**Trypsin Digestion of Protein Kinase C**—The post-AcA34 protein kinase C preparation in DEAE buffer containing 50% glycerol (0.05 mg/ml, ~6 units/ml) was digested with 1.0 μg of trypsin for 10 min at 30 °C. The digestion was stopped by the addition of 50–100 μg of soybean trypsin inhibitor and a 1:1 dilution with the same buffer containing 2 mg/ml ovalbumin. The enzyme was then kept at 4 °C and used within an hour, since further digestion and/or enzyme inactivation proceeded slowly upon incubation at 4 °C. Where indicated, the enzyme was first autophosphorylated for 3 min at 30 °C and then digested with trypsin. The digestion was stopped either as described above or by adding Laemmli PAGE sample buffer (14).

**DEAE Chromatography of Digestion Protein Kinase C**—Post-AcA34 protein kinase C was phosphorylated as described above, using [γ-32P]ATP. After 3 min, the reaction was stopped with EDTA and ATP (each 0.2 M), the samples were placed on ice, and Triton X-100 was added to give a final concentration of 0.5%. After 10 min at 4 °C, the conductivity of the samples was adjusted to ~0.8 mmho by dilution in DEAE buffer (~25-fold dilution). The samples were then applied to 1 ml pre-equilibrated DEAE columns. The columns were washed with 10 volumes of DEAE buffer, and the bound enzyme was eluted with a linear gradient of NaCl.

**Preparation of Monoclonal Antibodies**—Highly purified enzyme was injected into two Biozi mice (Naval Base Laboratories, Oakland, CA), and hybridomas were obtained by the method of Kohler and Milstein (15). The hybridoma media were screened for anti-protein kinase C activity by two methods: enzyme-linked immunosorbent assay and a screening method for antibodies which inhibits the enzymatic activity. Monoclonal antibodies 1.9 and 1.7, which interfere with the kinase activity, are negative in the enzyme-linked immunosorbent assay. Monoclonal antibodies 1.2 and 1.4 are positive in the enzyme-linked immunosorbent assay, but only monoclonal antibody 1.2 interacts with protein kinase C in immunoblot.

**Modulation of Protein Kinase C Activity by Monoclonal Antibodies**—50% of protein kinase C (0.15 units) or trypsin-treated protein kinase C (0.05 units, phospholipid- and Ca2+-independent), diluted in 20 mM Tris-HCl, pH 7.4, containing 2 mg/ml ovalbumin and 0.01% leupeptin and 1 μg/ml soybean trypsin inhibitor, were incubated with 20 μl of different monoclonal antibodies at the indicated concentration for 15 min at 30 °C. Phospholipid and Ca2+ or EGTA were added, and H1 histone phosphorylation or the phosphorylation of the substrates present in the enzyme preparation was measured under the standard conditions. When indicated, the amount of 32P incorporated into protein was analyzed on PAGE. The autoradiogram of the gels was scanned with a densitometer (E. C. Apparatus Corporation).

**RESULTS**

Previous studies on protein kinase C have reported that the protein phosphorylates itself (4, 16), but no detailed data have been published. Because of the importance of this finding, we proceeded to verify it independently but will only report one result in detail, because our studies confirm previous reports that protein kinase C autophosphorylates.

Briefly, the protein was purified to homogeneity and shown to phosphorylate a protein of the same molecular mass as itself, i.e., 80-kDa. The half-times for phosphorylation of the 80-kDa protein and histone phosphorylation were identical (Fig. 1). In order to establish whether an impurity copurifying and co-migrating with protein kinase C accounted for the 80-kDa band, the thermal inactivation of histone and 80-kDa phosphorylation were examined. By comparing heat sensitivities and the response to histones and self-phosphorylation, it was found that the protein kinase C is highly unstable and that histone phosphorylation and autophosphorylation are inactivated in parallel (Fig. 2). These studies revealed that the enzyme is most stable in the presence of high amounts of glycerol. For example, at 45 °C in 12.5% glycerol, about half of the enzymatic activity is lost within 2 min. In 50% glycerol, it takes 20 min. By varying the conditions over a wide range, it was always observed that inactivation of protein kinase C paralleled inactivation of histone phosphorylation.

An intriguing new phenomenon which also provides support for autophosphorylation was obtained by varying the concentration of the enzyme and observing the rate of autophosphorylation. Results are shown in Fig. 3. In a standard evaluation of the K_m of a protein, the enzyme is held constant in concentration while the substrate is varied up to saturating level. The kinetic analysis cannot be done in precisely this way for...
adjusted to a constant concentration of enzyme. From a number of phosphorylation would appear to be the maximum number under these conditions. However, by dilution one should obtain a second-order dependence on concentration if a monomeric enzyme is acting on itself. If one then divides by enzyme concentration, the phosphorylation would be first-order. Fig. 3 shows that the activity at various stages of dilution divided by the actual concentration of enzyme follows a plot equivalent to "zero-order" kinetics in a conventional enzyme assay, i.e. it indicates saturation by substrate when corrected for the concentration of enzyme. If the enzyme is truly a monomer, such a conclusion would mean that one part of the polypeptide chain was phosphorylating another part. A second alternative is also possible: the protein may exist as a polymer in which one subunit phosphorylates another subunit in the aggregate. This led us to a search for evidence of polymerization, which is also possible: the protein may exist as a polymer in which one subunit phosphorylates another subunit in the aggregate.

Using the number of radioactive phosphate incorporated and the number of molecules of enzyme, it is calculated that 2–3 molecules of phosphate/molecule of enzyme are incorporated. Analysis of tryptic digests reveals that the phosphates are donated primarily to the 37-kDa fragment (see below).

The Catalytic Fragment of Protein Kinase C—Protein kinase C is suggested to have two domains which are separated upon partial proteolysis: a hydrophobic domain, which allows the enzyme to interact with the membrane, and a catalytic domain (4). Partial digestion of protein kinase C by trypsin generates a catalytic fragment with an activity that is Ca²⁺, phosphatidyl serine- and diacylglycerol-independent (Fig. 4, lane b, in comparison to lane d, and Ref. 17, 18). Unlike the intact enzyme, the catalytic fragment of protein kinase C does not autophosphorylate (Fig. 4, lanes e and f versus g and h). This suggests that the fragment is either lacking the site for autophosphorylation or has a much lower affinity for this site. In order to study these possibilities, we first identified the phosphorylated protein kinase C fragments obtained by trypsin digestion of the intact prephosphorylated enzyme (Fig. 5). The amount of 80-kDa phosphoprotein band and the autophosphorylated protein kinase C are found to decrease with time of proteolysis, and concomittantly, 46-kDa and 37-kDa phosphoprotein bands both appear (Fig. 5). Additional protein kinase C fragment intermediates may be the ~60-, ~67-, and ~74-kDa phosphoproteins (Fig. 5b and data not shown) which also appear upon storage of protein kinase C (Fig. 5c). Enzyme stored at 4 °C before or after DEAE chromatography generates an additional major protein band of 74-kDa that is phosphorylated in the presence of phospholipid and calcium (Fig. 5c). The phosphorylated fragments obtained from this preparation by limited trypsin digestion are quite similar to the ones obtained from the intact enzyme (Fig. 5a), except that the stored enzyme yields a much higher amount of 74-kDa phosphoprotein and additional phosphopeptides below the 37-kDa one are obtained (Fig. 5d).

In order to identify which, if any, of the phosphorylated fragments shown in Fig. 5b is the catalytic fragment, phosphorylated intact protein kinase C is digested with trypsin, and the labeled fragments are separated by DEAE chroma-
tography and assayed for both kinase activity (Fig. 6C) and the presence of the phosphorylated fragments (Fig. 6D). In- tact phosphorylated protein kinase C elutes at 50-80 mM NaCl (Fig. 6A). Since the hydrophobic domain is removed, the catalytic fragment is expected to elute at a higher salt concentration. Indeed, kinase activity independent of phospholipid and Ca\(^{2+}\) elutes with about 250 mM NaCl (Fig. 6C). An autoradiogram analysis of these fractions after SDS-PAGE is shown in Fig. 6D and B for the digested enzyme and the intact one, respectively. The major phosphoprotein which elutes at high salt is the 46-kDa protein together with minor bands of 44 kDa and two of 60 and 57 kDa (Fig. 6D). Fractions containing the highest amount of 46-kDa phosphoprotein also have the highest kinase activity (Fig. 6C). This correlation suggests that the 46-kDa band (and possibly the 44-kDa band as well) are the catalytic fragments. No such correlation is found with the ~60 kDa peptides. The 37-kDa phosphoprotein is not recovered in any of the fractions, and more than one-third of the radioactive phosphopeptides are found in the column flow-through. If the 37-kDa fragment does not bind DEAE, it is in accordance with the hydrophobic nature expected of the regulatory domain. However, other possibilities cannot be excluded yet.

When the stored partially digested enzyme from Fig. 5d is applied to the DEAE column, the kinase activity elutes at a lower salt concentration than previously (150 mM versus 230 mM NaCl, in Figs. 7A and 6C, respectively), but higher than intact kinase (80 mM NaCl, Fig. 6A). The 44-, 74-, and 37-kDa phosphoproteins are found in fractions completely devoid of kinase activity (e.g. fractions 15–19, Fig. 7, A and B). Again, the 46-kDa phosphopeptide coelutes in the fractions which contain the highest amount of kinase activity (fractions 21–24, Fig. 7, A and C), supporting the previous suggestion that the catalytic fragment is indeed 46 kDa and that the other phosphorylated fragments are inactive.

In addition, evidence for the aggregation of activated protein kinase C is obtained from these experiments. The isolated 46-kDa catalytic fragment elutes from DEAE column at high salt concentration (230 mM NaCl, Fig. 6D), as expected for a protein which has its hydrophobic domain removed. The intact enzyme elutes at 80 mM NaCl (Fig. 6B). However, after partial digestion, the catalytic (46-kDa) and the putative regulatory (37-kDa) fragments coelute at intermediate salt concentration (150 mM NaCl, Fig. 7B), suggesting that the fragments are associated. This can occur if, in an enzyme aggregate, one or more of the kinase molecules remain intact. In this case, some of the fragments can be held together in the aggregate and therefore will coelute at an intermediate salt concentration.

The Effect of Autophosphorylation—To determine the physiological significance of the autophosphorylation, the $K_m$ and $V_{max}$ of the phosphorylated and nonphosphorylated enzyme were determined. Autophosphorylation increases enzymatic activity when using H1 histone protein or potential substrates such as the ~20- and ~45-kDa proteins, shown to be phosphorylated by protein kinase C (data not shown). In experiments such as these, we found that autophosphorylation results in a slightly decreased $K_m$ for histone (2.3 ± 0.2 and 1.4 ± 0.1 μM, respectively, for nonphosphorylated and phosphorylated protein kinase C). A decrease in the $V_{max}$ from 5.8 ± 0.2 to 3.6 ± 0.2 units/ml for the nonphosphorylated and autophosphorylated enzyme, respectively, is observed. Thus autophosphorylation inhibits activity at histone concentration close to the $K_m$, but stimulates it at low concentrations. The presence of excess protein substrate such as H1 histone inhibits protein kinase C autophosphorylation (Fig. 8, b versus a). Significant inhibition occurs at approximately 1 μM H1 histone, just below the $K_m$ value for this substrate.

As shown above, protein kinase C undergoes chemical modification, autophosphorylation. We have verified that the decrease in $K_m$ for H1 histone is a result of this phosphorylation rather than precomplexing of the enzyme with ATP in the following experiment. Enzyme is allowed to incubate with radiolabeled ATP. At the indicated time, H1 histone and excess of unlabeled ATP are added, and the transfer of radiolabeled phosphate to H1 histone is determined. We found that under these conditions H1 histone is not radiolabeled, indicating that the change in kinetic parameters upon autophosphorylation is not a result of ATP being precomplexed with the enzyme.

The Effect of Enzymatic Digestion—As demonstrated (Fig. 4), a partial digestion of protein kinase C generates Ca\(^{2+}\)- and phospholipid-independent kinase activity, and longer digestion results in kinase inactivation. The trypsin-digested protein kinase C has a lower affinity for H1 histone ($K_m = 9.8 \pm 0.8 \mu M$ compared to $1.7 \pm 0.3 \mu M$ for nondigested protein kinase C), whereas $V_{max}$ changes very little ($V_{max} = 6.5 \pm 0.2$ and $5.7 \pm 0.7$ units/ml for digested and nondigested protein kinase C, respectively).

**Anti-Protein Kinase C Monoclonal Antibodies**—We
have generated monoclonal antibodies against highly purified protein kinase C. Of the four antibodies described here, three affect the activity of the enzyme. Monoclonal antibodies 1.4 and 1.9 inhibit its activity (Fig. 9). However, the two antibodies do not bind to the same site, since monoclonal activity 1.9 can inhibit both the intact enzyme and the catalytic fragment, whereas monoclonal antibody 1.4 inhibits only the intact enzyme (Fig. 9). The lower inhibition of the digested enzyme obtained by monoclonal antibody 1.9 is probably due to the presence of some inactive kinase fragments which retain their antigenicity and therefore compete for antibody 1.9 binding. Monoclonal antibody 1.2, which reacts only with the intact enzyme in immunoblot, does not affect the activity of either the intact or the catalytic fragment (Fig. 9, left panel).

The effect of another monoclonal antibody, 1.7, is found to be different from monoclonal antibodies 1.4 and 1.9. H1 histone phosphorylation increases by 70% when the enzyme is incubated with monoclonal antibody 1.7. 40% increase is found when the enzyme is activated by phospholipids prior to the addition of monoclonal antibody 1.7, and the activating effect of monoclonal antibody 1.7 is abolished if the enzyme is first activated by both phospholipids and calcium. The same effect of monoclonal antibody 1.7 is found also on another protein kinase C substrate present in the enzyme preparation (Fig. 10). In this assay, after preincubation of the enzyme with the antibody, the enzyme's activators and radiolabeled ATP are added. Although the phosphorylation of the...
protein substrate increases as described earlier for H1 histone phosphorylation of the 67-kDa protein, a protein kinase C fragment (Fig. 5 and Ref. 19) and the autophosphorylation decreases (Fig. 10). Unlike the 74-kDa fragment of protein kinase C, which is inactive (Fig. 7, a and b), the 67-kDa fragment found in some of the enzyme preparations is active (data not shown and Ref. 19). The effect of monoclonal antibody 1.7 on the phosphorylation of the 43-kDa substrate is most apparent in the presence of calcium with or without phosphatidylyserine and diacylglycerol (Fig. 10, A and B).

However, the inhibitory effect on autophosphorylation is found also in the absence of calcium (Fig. 10, C and D). This differential effect of the antibody on self-phosphorylation and substrate phosphorylation may indicate that the antibody replaces the activating effect of the autophosphorylation, possibly by inducing the aggregation of the enzyme. However, other explanations are not excluded.

**DISCUSSION**

**Autophosphorylation and Zero-order Kinetics of Protein Kinase C**—Protein kinase C has represented a fascinating puzzle, because it has both strong similarities and very important differences from the second messenger systems that have been uncovered before it. It appears to be appreciably more complex than the cyclic AMP and Ca²⁺ second messenger systems. The regulation of its activity has implications not only for normal signal transduction, but also for tumorogenesis (5). Yet protein kinase C shares many of the properties of the other systems in the sense of pleotropic effects (e.g. 20-22) and a feedback relationship to other cellular processes (e.g. 5, 23-26). To unravel its basic function will require further understanding of the enzyme, its kinetic and biological properties. Some of these have been clarified above.

In the first place, it is clear that the proteolytic cleavage separates the catalytic domain of the enzyme from a more hydrophobic domain (18). The hydrophobic domain must play a regulatory role, since the inhibition of this domain can be released by calcium and phospholipid in a reversible manner for the holoenzyme or in an irreversible manner by proteolytic cleavage.

Superimposed on this regulation by allosteric effectors or cleavage is another modification by phosphorylation (Fig. 3), which apparently enhances the activity of the enzyme. The phosphorylation appears to place phosphate groups on both the catalytic and the regulatory domains (Fig. 5). The exact function of each of these phosphate groups is not yet clear, but it is apparent that both the $K_\text{m}$ and $V_\text{max}$ values of the proteins so modified are changed. The effect of this covalent modification is to activate the enzyme, in contrast to the covalent modification of other receptors which desensitizes the enzyme. Monoclonal antibody 1.7 is more complex, and its site of affect the activity (in either way) and therefore is a good marker for the enzyme in any conformation. The effect of this antibody is shown, suggesting the presence of dimeric protein kinase C. Although evidence for aggregation is presented, ongoing studies reveal that monomeric protein kinase C is capable of self-phosphorylation.

It is interesting to note that in several instances, in vivo stimulation of protein kinase C results in the phosphorylation of an 80-kDa protein, presumably the autophosphorylated protein kinase C (e.g. Refs. 28, 29). Moreover, prolonged activation of the enzyme by phorbol esters in platelets results in the generation of the catalytic fragment (30). These observations indicate that regulation by allosteric effects or cleavage is physiological and therefore may play an important role in the tuning of the activity of this important second messenger system.

**Monoclonal Antibodies**—The monoclonal antibodies add further insight to the structural properties of the protein. Monoclonal antibody 1.9 appears to act near the active site of the enzyme, since it can inhibit both the intact enzyme and the catalytic fragment, and its inhibitory activity is reduced by an excess of ATP. On the other hand, monoclonal antibody 1.4 inhibits only the intact enzyme and thus suggests that it acts on the conformation of the protein and not directly at the active site. Monoclonal antibody 1.2, which reacts only with the intact enzyme (as shown in immunoblot), does not affect the activity (in either way) and therefore is a good marker for the enzyme in any conformation. The effect of monoclonal antibody 1.7 is more complex, and its site of interaction is not clear as yet. A scheme of the protein kinase C domains and the putative sites for the antibody binding is given in Fig. 11. The differential specificity of the monoclonal antibodies will enable us to analyze the regulation of protein kinase C activity in vivo.

This study suggests multiple controls on protein kinase C. Thus, the system is not only pleiotropic in the sense that the protein kinase C works on a number of different substrates, but it also apparently is regulated in activity by a number of different factors impinging on the enzyme. The idea that the second messenger system is simply taking information from a stimulus and transmitting it to some distant site across the cell appears untenable. The messenger system itself obviously undergoes feedback regulation of a number of types, and some of these are indicated by the properties of the protein itself.

**Acknowledgment**—The authors are grateful for help in the experimental work by Denise Pollard-Knight, Alex Karu, Andrew Flint, and Alexandra Newton.

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2 A. C. Newton and D. E. Koshland, manuscript in preparation.
Note Added in Proof—Since submission of this manuscript, Huang et al. (J. Biol. Chem. 1986) 261, 12134-12140 and Lee and Bell (J. Biol. Chem. 1986) 261, 14867-14870 have reported similar findings in regard to the autophosphorylation and the 37-kDa tryptic fragment.

REFERENCES