A structural feature shared by many protein kinases is the requirement for phosphorylation of threonine or tyrosine in the so-called activation loop for full enzyme activity. Previous studies by several groups have indicated that the isoforms α, β, and γ of protein kinase C (PKC) are synthesized as inactive precursors and require phosphorylation by a putative "PKC kinase" for permissive activation. Expression of PKCα in bacteria resulted in a nonfunctional enzyme, apparently due to lack of this kinase. The phosphorylation sites for the PKC kinase in the activation loop of PKCα and PKCβII could be identified as Thr497 and Thr500, respectively. We report here that PKCδ, contrary to PKCα, can be expressed in bacteria in a functional form. The activity of the recombinant enzyme regarding substrate phosphorylation, autophosphorylation, and dependence on activation by 12-O-tetradecanoylphorbol-13-acetate as well as the Km values for two substrates are comparable to those of recombinant PKCδ expressed in baculovirus-infected insect cells. By site-directed mutagenesis we were able to show that Thr505, corresponding to Thr497 and Thr500 of PKCα and PKCβII, respectively, is not essential for obtaining a catalytically competent conformation of PKCδ. The mutant Ala504 can be activated and does not differ from the wild type regarding activity and several other features. Ser504 cannot take over the role of Thr505 and is not prerequisite for the kinase to become activated, as proven by the unaffected enzyme activity. Previous studies by several groups have indicated that at least this PKC isoenzyme differs from the Iso 1 regarding permissive activation. Expression of PKCδ was purified to homogeneity. The phosphorylation of threonine or any other functional recombinant PKC isoenzyme in bacteria has not been described yet.

PKCδ is a ubiquitously expressed PKC isoform (12) and exhibits some unique properties. The tyrosine kinase c-Src selectively phosphorylates the type δ PKC isoform in vitro. The tyrosine phosphorylation induces a modification of PKCδ activity exhibiting some substrate selectivity (13). Tyrosine phosphorylation of PKCδ could be demonstrated also in vivo (14, 15). In addition to the substrate specificity acquired by tyrosine phosphorylation, PKCδ appears to possess an intrinsic substrate specificity, for example, toward the elongation factor 1α and an elongation factor 1α peptide (16). PKCδ is autophosphorylated to a much higher degree than the other isoenzymes, and in contrast to other kinases, including PKC isoforms, it is able to accept GTP as a phosphate donor for autophosphorylation (17). Based on results obtained with cells overexpressing PKCδ, some role of this PKC isoform in growth suppression and induction of differentiation has been suggested (18–20). The finding that PKCδ is lost from immortalized human keratinocytes after stable transfection with a c-Ha-ras oncogene also points to this possible function (21).

Here we report on another, possibly unique, feature of PKCδ. Contrary to PKCα (22–24), PKCδ could be expressed in bacteria in a functional form. Moreover, we were able to demonstrate by site-directed mutagenesis that phosphorylation by a putative "PKC kinase" of Thr505, unlike that of the corresponding Thr497 and Thr500 in PKCα (24) and βII (25), respectively, is not essential for a permissive activation of PKCδ.

**EXPERIMENTAL PROCEDURES**

**MATERIALS—**TPA was supplied by Dr. E. Hecker (German Cancer Research Center). Go 6976 and Go 6983 were kindly provided by Goedecke A.G. (Freiburg, Germany). The rat PKCδ full-length cDNA clone (3000 base pairs) and the recombinant baculovirus containing the sequence coding for PKCδ were generously given by Dr. C. Polke (University of Würzburg, Würzburg, Germany) and Dr. S. Stabel (Max-Planck-Laboratorium, Cologne, Germany). The pseudosubstrate-related peptide δ (MNRRGSIKQAKI) was synthesized by Dr. R. Pipkorn (German Cancer Research Center). Other materials were bought from the following companies: bovine brain t-α-phosphatidylycerine (PS) and histone III-S (lysine-rich fraction from calf thymus) from Sigma; [γ-32P]ATP (specific activity, 5000 Ci/mmol) from Hartmann Analytic

**Phosphorylation of Protein Kinase Cδ (PKCδ) at Threonine 505 Is Not a Prerequisite for Enzymatic Activity**

**EXPRESSION OF RAT PKCδ AND AN ALANINE 505 MUTANT IN BACTERIA IN A FUNCTIONAL FORM**

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Bacterial Expression of PKC

Polymerase Chain Reaction Amplification and Cloning of Wild Type and Mutant PKCα cDNAs—For the construction of a PKCα full-length cDNA with an Ndel restriction site at the initiation signal ATG we used an EcoRI restriction site behind the stop codon TGA, the following oligonucleotide primers were used: 5′-AAA GGA TCC CAT ATG GCA CCG TTC CGT CGC-3′ as 5′-primer and 5′-TCT GGG AAT TCA CTA CTA TCC GAA TGG TCT CTC-3′ as 3′-primer (synthesized by W. Weinig, German Cancer Research Center). For polymerase chain reaction amplification (cycle profile: 94°C/5 min; 10°C/1 min, 76°C/1 min; 72°C/1 min), a rat PKCα full-length cDNA—containing the full-length cDNA of rat PKCα (PKCα wild type) and were grown as described under “Experimental Procedures.” Bacteria were transformed with 1 ml of buffer I and buffer I-insoluble proteins dissolved in 1 ml of sample buffer containing 1% SDS. Soluble (s) and insoluble (b) proteins (3 μl each) were separated by SDS-PAGE. PKCα (arrow) was identified by immunoblotting with a PKCα-specific antibody and an alkaline phosphatase-conjugated goat anti-rabbit IgG as secondary antibody. Molecular masses were determined from the standard proteins myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), and ovalbumin (45 kDa). B, comparison of the concentration of recombinant PKCα in extracts from bacteria and baculovirus-infected insect cells. Extracts from bacteria that were transformed with the plasmids pET28wt or pET28Ala505 and produced the PKCα wild type (wt) or the PKCαAla505 mutant (Ala505) and extracts from baculovirus-infected Sf9 insect cells expressing PKCα wild type (Baculo) were diluted as indicated (μg protein). On separation of the extracted proteins by SDS-PAGE the amount of PKCα was estimated by immunoblotting (see A).

Protein Kinase Cα Does Not Need Thr505 for Enzymatic Activity

Figure 1. A, expression of PKCα in E. coli BL21(DE3)pLysS cells. Bacteria were transformed with the plasmid pET28 alone or the plasmid pET28tht (wt) containing the full-length cDNA of rat PKCα (PKCα wild type) and were grown as described under “Experimental Procedures.” Bacteria were transformed with 1 ml of buffer I and buffer I-insoluble proteins dissolved in 1 ml of sample buffer containing 1% SDS. Soluble (s) and insoluble (b) proteins (3 μl each) were separated by SDS-PAGE. PKCα (arrow) was identified by immunoblotting with a PKCα-specific antibody and an alkaline phosphatase-conjugated goat anti-rabbit IgG as secondary antibody. Molecular masses were determined from the standard proteins myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), and ovalbumin (45 kDa). B, comparison of the concentration of recombinant PKCα in extracts from bacteria and baculovirus-infected insect cells. Extracts from bacteria that were transformed with the plasmids pET28wt or pET28Ala505 and produced the PKCα wild type (wt) or the PKCαAla505 mutant (Ala505) and extracts from baculovirus-infected Sf9 insect cells expressing PKCα wild type (Baculo) were diluted as indicated (μg protein). On separation of the extracted proteins by SDS-PAGE the amount of PKCα was estimated by immunoblotting (see A).

(Braunschweig, Germany), recombinant human protein kinase c-Src from Upstate Biotechnology Inc. (Lake Placid, NY), mouse monoclonal anti-phosphotyrosine antibodies (PY20) from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), peroxidase- and alkaline phosphatase-conjugated goat anti-rabbit and anti-mouse antibodies from Dianova (Hamburg, Germany), and ECL reagents from Amersham Corp. Expression vector pET28 and Escherichia coli strain BL21(DE3)pLysS were from AGS GmbH (Heidelberg, Germany), nickel-nitrilo-triacetic acid resin from Qiagen GmbH (Hilden, Germany), T7-Sequencing™ kit from Pharmacia Biotech Inc., and Pwo DNA polymerase from Boehringer Mannheim.

Polymerase Chain Reaction Amplification and Cloning of Wild Type and Mutant PKCα cDNAs—For the construction of a PKCα full-length cDNA with an Ndel restriction site at the initiation signal ATG we used an EcoRI restriction site behind the stop codon TGA, the following oligonucleotide primers were used: 5′-AAA GGA TCC CAT ATG GCA CCG TTC CGT CGC-3′ as 5′-primer and 5′-TCT GGG AAT TCA CTA CTA TCC GAA TGG TCT CTC-3′ as 3′-primer (synthesized by W. Weinig, German Cancer Research Center). For polymerase chain reaction amplification (cycle profile: 94°C/5 min; 10°C/1 min, 76°C/1 min; 72°C/1 min), a rat PKCα full-length cDNA clone of 3000 base pairs served as a source of bacterial recombinant His-tagged PKCα. The insoluble fraction was resuspended in SDS sample buffer and boiled for 5 min before application to PAGE.

Partial Purification of Recombinant His-tagged PKCα—Partial purification of soluble PKCα was achieved by metal chelate affinity chromatography of the bacterial extract under native conditions using nickel-nitrilo-triacetic acid resin and following the manufacturer’s recommendation. Bound proteins were eluted with imidazole (100, 150, 200, 250, and 500 mM). PKCα was detected in the 100 mM imidazole fraction by immunoblotting and assaying PS- and TPA-stimulated kinase activity.

Recombinant PKCα from Baculovirus-infected Insect Cells—Sf9 cells were infected with the recombinant baculovirus, and cells were extracted as described previously (27).

Protein Kinase Assay—Phosphorylation reactions were carried out in a total volume of 100 μl containing buffer C (50 mM Tris-HCl, pH 7.5, 10 mM β-mercaptoethanol), 4 mM MgCl2, 10 μg of PS, 100 nM TPA, 2 μg of pseudosubstrate-related peptide δ or 30 μg of histone III-S as substrate, 10 μl of bacterial extracts diluted as indicated in figure legends, and 37 μM ATP containing 1 μCi [γ-32P]ATP. In some experiments, PS and TPA were omitted, and in some others, G6 6976 or G6 6983 at concentrations indicated in figure legends were added. After incubation for 7 min at 30°C, the reaction was terminated by transferring 50 μl of the assay mixture onto a 20-mm square piece of phosphocellulose paper (Whatman p81). After washing the paper three times in deionized water and twice in acetone, radioactivity was determined by liquid scintillation counting. 1 unit of kinase activity equals 1 nmoI of phosphate incorporated into substrate/min.
The bacterial recombinant PKCδ was shown to be catalytically active in the absence of PS and TPA or in the presence of PS alone. An extract of bacteria transformed with the pET28 vector alone, the pET28 vector alone, or the expression vector pET28ΔaAla505 containing the PKCδ-mutant cDNA did not show any TPA-inducible kinase activity. To be able to compare the kinase activity of recombinant PKCδ from bacteria with that from baculovirus-infected insect cells, nearly equal amounts of PKCδ had to be applied to the kinase assay. This was achieved by diluting the insect cell extract 1:11 to approximately equalize the concentration of PKCδ with Coomassie Blue but only by immunoblotting. The concentration of PKCδ was determined with the protein dye reagent concentrate (according to the method of Bradford; Ref. 53) from Bio-Rad, using bovine serum albumin as standard.

**RESULTS**

At transformation with the expression vector pET28 wt containing the full-length cDNA of rat PKCδ, although not with the pET28 vector alone, the E. coli cells BL21(DE3)pLysS produced PKCδ, as demonstrated by immunoblotting of bacterial proteins with a PKCδ-specific antibody (Fig. 1A). A portion of the ectopically expressed rat PKCδ was soluble in a buffer without detergent (here termed bacterial extract). The insoluble fraction, present in the bacteria probably in the form of inclusion bodies, was dissolved in sample buffer containing 1% SDS. Slow growth of the bacteria at 24 °C was expected to result in an increased portion of active recombinant enzyme.

PKCδ could not be detected on staining of bacterial proteins with Coomassie Blue but only by immunoblotting. The concentration of PKCδ in the bacterial extract was approximately one-third of that in an extract of insect cells that produced PKCδ on infection with a recombinant baculovirus (Fig. 1B). The bacterial recombinant PKCδ was found to be enzymatically active. As shown in Fig. 2, histone III-S was phosphorylated by the recombinant enzyme in the bacterial extract in a TPA-dependent manner. Only weak incorporation of phosphate occurred in the absence of PS and TPA or in the presence of PS alone. An extract of bacteria transformed with the pET28 vector alone served as a control and did not show any TPA-inducible kinase activity. To be able to compare the kinase activity of recombinant PKCδ from bacteria with that from baculovirus-infected insect cells, nearly equal amounts of PKCδ had to be applied to the kinase assay. This was achieved by diluting the insect cell extract 1:11 (final concentration of protein in the assay, 0.08 mg/ml) and the bacterial extract 1:4 (final concentration of protein in the assay, 0.2 mg/ml), according to the data on the concentration of PKCδ in both extracts (see Fig. 1B).

The activity of PKCδ produced by the bacteria proved to be comparable to that of PKCδ expressed in insect cells (Fig. 2). Enzyme activity was measured with the substrates histone III-S (Fig. 2) and pseudosubstrate-related peptide δ as substrates. Moreover, no significant difference between both enzymes could be detected regarding either activation by TPA (Fig. 2) or specific activities and $K_m$ values for the pseudosubstrate-related peptide δ and histone III-S (Table I).

**Table I.** Specific activities and $K_m$ values of bacterial recombinant PKCδ wt and PKCδAla505 mut as well as of recombinant PKCδ expressed in baculovirus-infected insect cells (Baculo).

| Peptide δ | Histone III-S |
|-----------|---------------|
|           | $K_m$ Spec. act. | $K_m$ Spec. act. |
| Baculo     | μM units/mg | μM units/ml | units/mg |
| wt         | 13 3.95 125 1.1 |
| Ala505     | 6 2.0 140 1.5 |

* Spec. act., specific activity.
peptide δ as substrate, and 1.1 and 1.5 units/mg, respectively, with histone III-S as substrate) as well as the \( K_m \) values (5 and 6 \( \mu M \), respectively, for peptide δ, and 125 and 140 \( \mu M \), respectively, for histone III-S) were essentially the same (Table I).

For a more detailed characterization of bacterial recombinant PKCδ wild type and PKCδAla\(^{505} \) mutant (Ala505), we determined the enzyme activity of PKCδ in the diluted extracts (10 \( \mu l \), see Fig. 2) in the presence or absence of PS and TPA and is given as percentage of control (activity in the absence of inhibitor).

Protein Kinase Cδ Does Not Need Thr\(^{505} \) for Enzymatic Activity

**FIG. 3.** Autophosphorylation of bacterial recombinant PKCδ wild type and PKCδAla\(^{505} \) mutant. 10 \( \mu l \) of the diluted extracts (see Fig. 2) from bacteria transformed with the plasmids pET28, pET28wt (wt), or pET28Ala\(^{505} \) (Ala505) were phosphorylated with \([\text{\textsuperscript{32}P}]\text{ATP in vitro} \) in the presence or absence of PS and TPA as described under “Experimental Procedures.” Phosphorylated proteins were separated by SDS-PAGE and visualized by autoradiography. The location of PKCδ, as identified by immunoblotting, is indicated by an arrow.

**FIG. 4.** Phosphorylation of bacterial recombinant PKCδ wild type (wt) and PKCδAla\(^{505} \) mutant (Ala505) with the tyrosine kinase Src. Diluted bacterial extracts (10 \( \mu l \), see Fig. 2) were phosphorylated with 6 units of c-Src as described under “Experimental Procedures.” PKCδ was identified by immunoblotting with an anti-PKCδ antibody (A). After stripping of the blot, tyrosine-phosphorylated proteins, including PKCδ, were identified using an anti-phosphotyrosine antibody (B), as described under “Experimental Procedures.” As secondary antibodies goat anti-rabbit and goat anti-mouse peroxidase-conjugated antibodies were used for the first and second blots, respectively. The location of PKCδ is indicated by arrows (note the shift on tyrosine phosphorylation).

**FIG. 5.** Suppression of the kinase activity of bacterial recombinant PKCδ wild type (wt) and PKCδAla\(^{505} \) mutant (Ala505) by the staurosporine-derived inhibitors Gö 6976 (A) and Gö 6983 (B). The enzyme activity of PKCδ in the diluted extracts (10 \( \mu l \), see Fig. 2) was determined in the absence or presence of the inhibitors (concentrations as indicated) by the kinase assay, as described under “Experimental Procedures.” Kinase activity was determined in the presence of PS and TPA and is given as percentage of control (activity in the absence of inhibitor).
Finally, we studied the inhibition of both types of recombinant PKCδ by the staurosporine-related protein kinase inhibitors Gö 6976 and Gö 6983, which are specific for PKC. The two inhibitors are known to differ significantly in their capacity to suppress PKCδ, with IC_{50} values of 1 μM and larger for Gö 6976 and 10–100 nM for Gö 6983 (29–31). A similar difference in inhibitory potency was observed with the bacterial recombinant PKCδ wild type and mutant (Fig. 5). Suppression by Gö 6976 occurred, with IC_{50} values in the range of 10–30 μM and by Gö 6983 in the range of 25 nM.

We could not entirely exclude the possibility that in PKCδ Ser^504 might be able to take over the role of Thr^505 and might serve as an essential phosphorylation site, such as Thr^497 and Thr^500 of PKCδ and β_1, respectively. Therefore, we expressed two other mutants of PKCδ, PKCδAla^504 and PKCδAla^504/Ala^505, in the bacteria, in which serine 504 alone or serine 504 and threonine 505 were exchanged for alanine. As shown in Fig. 6, both mutants actively phosphorylated the pseudosubstrate-related peptide δ as substrate, as described under “Experimental Procedures.” Values are the means of three determinations (bars, ±S.E.).

In preliminary experiments bacterial recombinant PKCδ (wild type and mutants) containing a short His tag could be partially purified by affinity chromatography on a nickel-ni-trilo-triacetic acid resin. On elution from the column with 100 mM imidazole the purity of the enzymes was around 20%, as estimated from Coomassie Blue-stained SDS-polyacrylamide gels. The specific activities (the mean of two experiments), as determined with histone III-S as substrate, were 46.2 ± 6.2 units/mg protein (wild type), 59.5 ± 13.3 units/mg protein (PKCδAla^505), and 54.2 ± 6.1 units/mg protein (PKCδAla^504/Ala^505).

**DISCUSSION**

Fabbro and co-workers (32) provided the first evidence of PKCa being synthesized as an inactive nonphosphorylated precursor, which is at first converted to a transient and finally to a “mature” phospho form. Further studies by this and another group showed that bacterial expression of PKCδ results in a recombinant protein devoid of kinase activity (22–24). It was suspected that phosphorylation by another protein kinase is necessary for PKCδ to gain the ability of being activated, and that bacteria lack this putative PKC kinase. According to our results, PKCδ can be expressed in bacteria in a functional form. Agreeing with previous reports (22–24), we were unable to express enzymatically active PKCδ in bacteria using the same expression vector and the same conditions as for the expression of PKCδ (data not shown). The activity of bacterially expressed PKCδ regarding substrate phosphorylation and TPA dependence as well as its K_m values for two substrates are comparable to those of recombinant PKCδ expressed in baculovirus-infected insect cells. Moreover, partial purification of recombinant PKCδ from bacterial extracts by one-step affinity chromatography yields an enzyme with a specific activity (46.2 units/mg protein) comparable to that of partially purified native PKCδ from porcine spleen, which previously was found to be 15 units/mg protein on the second purification step (phenyl-Sepharose) and 54 units/mg protein on the third purification step (protease-arasan; see Ref. 7). As the partially purified bacterial enzyme is around 20% pure, its specific activity is also in good agreement with the specific activity of the native enzyme purified to homogeneity from porcine spleen (304.2 units/mg protein; Ref. 7). Provided that the bacteria strain used in our studies indeed lacks the PKC kinase, these results indicate that PKCδ does not require phosphorylation by this
kinase to become a functional enzyme. Some protein kinases are able to autophosphorylate during bacterial expression by a presumably cotranslational intermolecular phosphorylation (33).

The sites phosphorylated by another kinase were identified in bovine PKCα (24) and rat PKCβII (25) as Thr497 and Thr500, respectively. These threonines are located in an activation loop that is also crucial for the regulation of other protein kinases (34–39). Replacement of these critical residues in PKCαs (24) or PKCβII (25) with a neutral, nonphosphorylatable residue results in kinases that cannot be activated. The corresponding site in rat PKCδ is Thr505. Bacterial expression of a PKCδ mutant containing alanine in position 505 yields a fully functional kinase. The mutant does not differ from the wild type in many respects, such as effectiveness of expression in bacteria, TPA-stimulated kinase activity, K_m values for substrates, autophosphorylation, tyrosine phosphorylation by Src, and inhibition by staurosporine-related inhibitors. This clearly demonstrates that functional PKCδ can be expressed in bacteria, and that Thr505, contrary to Thr497 and Thr500 in PKCα and βII, respectively, is not a critical site for permissive activation of PKCδ. As Ser504 is another amino acid that can be phosphorylated in this region of PKCδ, we wished to exclude the possibility that this residue might be replaced by Thr505 as a phosphorylation site for the putative PKC kinase and thus as the critical site for permissive activation. Exchange of Ser504 alone or both Ser504 and Thr505 for alanine does not result in any loss of kinase activity. Partial purification of the bacterial PKCδ mutants Ala505 and Ala504/Ala505 by affinity chromatography yields enzymes with specific activities of 59.5 and 54.2 units/mg protein, respectively, which are comparable to those of partially purified bacterial wild type PKCδ and native PKCδ from porcine spleen (see above). This proves that neither Thr505 nor Ser504 is essential for gaining a catalytically competent conformation of PKCδ. As each PKC isoenzyme known so far contains threonine in a position corresponding to positions 497 and 500 of PKCα and βII, respectively, the findings regarding the critical role of this threonine residue for the activation process of PKCδ and βII have been thought to be valid for all PKC isoenzymes (24, 40). According to our results, however, at least the isotype δ is an exception to this apparent rule. PKCδ phosphorylation is not required when a neutral, nonphosphorylatable amino acid instead of this threonine residue.

In accordance with reports on a stepwise phosphorylation of PKCαs (32, 41, 42) and on the in vitro phosphorylation sites of PKCβII (40, 43), Newton and co-workers (40, 44) have shown that phosphorylation of threonine 500, putatively by the PKC kinase, enables PKCβII to autophosphorylate at Thr641 and Ser660. Phosphorylation at Thr641 replaces the requirement for phosphate on Thr500 and stabilizes the functional form of the enzyme. A report by Zhang et al. (45) indicates that in PKCβII phosphorylation of Thr642 (corresponding to Thr641 in PKCβII) is an early event in the processing of the newly synthesized enzyme and is required for enzymatic functioning. Very recently, Bornancin and Parker (46) reported that phosphorylation of Thr638 of PKCα (corresponding to Thr638 of PKCδ and Thr641 of PKCβII) is not required for the catalytic function of the enzyme per se, but serves to control the duration of activation by regulating the rate of dephosphorylation and inactivation of the protein. This is achieved through the cooperative interaction between Thr638 and the catalytic core site, Thr497. It is conceivable that PKCδ also requires autophosphorylation of the corresponding site, i.e. Ser643, for stabilization of a catalytically competent conformation. As indicated by our results, however, PKCδ is able to autophosphorylate without having been phosphorylated by another kinase. As a consequence, a putative regulation of this PKC kinase would not affect PKCδ, and thus PKC kinase might be a target for a differential regulation of PKC isoenzymes. Identification of the in vitro phosphorylation sites of PKCδ should allow an answer to the question of whether any of the three in vitro phosphorylation sites found in PKCβII (40, 43) play a role also in PKCδ.

In the crystal structure of cAPK (47, 48) the diatomic phosphophoryl group of Thr197-P in the activation loop neutralizes a negatively charged residue from outside the catalytic core of PKCδ (40, 43) play a role also in PKCδ, we exchanged the side chains in the crystal structure of the catalytic core of PKCδ for those of PKCδ according to the alignment of Hanks and Quinn (49) while keeping the backbone unchanged (Fig. 7). Two of the side chain interactions with Thr197-P in cAPK are not possible in PKCδ; His87 from the small lobe is replaced by Cys399 in PKCδ, and Thr195 is replaced by Ala505. A cysteine in the position equivalent to His87 of cAPK is also conserved in PKCδ and PKCβII, which both require phosphorylation at Thr497 and Thr500, respectively, for activity. The basic residues Arg165 and Lys189 of cAPK, corresponding to Arg165 and Lys189 of PKCδ, are both conserved in PKCδ. It is likely that Arg165, which precedes the catalytic base, and perhaps Lys189 are conserved in protein kinases and thus also in PKCδ for similar functions. Both residues form salt bridges to Thr197-P in cAPK. The contact to Arg165 may directly promote the correct assembly of the active site by controlling the orientation of the catalytic base Asp166 via its peptide backbone, whereas the contact to Lys189 may help in correctly positioning the metal binding loop, i.e. essentially Asp184. However, from this model it is not apparent how in PKCδ the dispensable Thr505-P may be functionally substituted. Several possibilities can be discussed to explain the observed Thr505-independent activity of PKCδ. If an ionic interaction at this site is also needed for PKCδ, it could be provided by a bound ion, similarly as in casein kinase 1 (50, 51). On the other hand, a negatively charged residue from outside the catalytic core might reach into the activation site. Another possibility is that the PKCδ activation loop, which contains the three-residue insert Gly589–Glu590–Asn591, folds back to orient the Glu590 carboxylate in a position where it can interact with Arg165 and Lys189. Finally, nonionic interactions with Arg165, similarly to mammalian casein kinase 1 (51), are conceivable. The question remains why residue 505 is conserved as a threonine in PKCδ. The fact that Thr505 is dispensable for the permissive activation of PKCδ does not exclude its phosphorylation for other purposes, such as protein-protein interaction, as indicated in the interaction of cAPK catalytic and regulatory subunits (52), or enzyme inactivation.

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