PfPKB, a Novel Protein Kinase B-like Enzyme from Plasmodium falciparum

I. IDENTIFICATION, CHARACTERIZATION, AND POSSIBLE ROLE IN PARASITE DEVELOPMENT*

Extracellular signals control various important functions of a eukaryotic cell, which is often achieved by regulating a battery of protein kinases and phosphatases. Protein Kinase B (PKB) is an important member of the phosphatidylinositol 3-kinase-dependent signaling pathways in several eukaryotes, but the role of PKB in protozoan parasites is not known. We have identified a protein kinase B homologue in Plasmodium falciparum (PfPKB) that is expressed mainly in the schizonts and merozoites. Even though PfPKB shares high sequence homology with PKB catalytic domain, it lacks a pleckstrin homology domain typically found at the N terminus of the mammalian enzyme. Biochemical studies performed to understand the mechanism of PfPKB catalytic activation suggested (i) its activation is dependent on autophosphorylation of a serine residue (Ser-271) in its activation loop region and (ii) PfPKB has an unusual N-terminal region that was found to negatively regulate its catalytic activity. We also identified an inhibitor of PfPKB activity that also inhibits P. falciparum growth, suggesting that this enzyme may be important for the development of the parasite.

Plasmodium falciparum, a unicellular protozoan responsible for the most lethal form of human malaria, has re-emerged as a leading cause of mortality in developing countries, especially in the population of young children age 5 and under. Widespread drug resistance exacerbates the problem and limits our options for effective malaria control. Current efforts to produce effective vaccines have not yet resulted in any significant success. Identification of drugs that interfere with parasite development could be a useful way to inhibit parasite growth in humans. Detailed knowledge of molecular mechanisms that control the life cycle of malaria parasite could provide crucial information needed to achieve this goal.

The life cycle of the malaria parasite is a complex but a well synchronized series of events. After invasion of the erythrocytes, the parasite can either propagate asexually or undergo sexual differentiation. The role of extracellular signals and molecular events in parasite life cycle are not well understood. It is well known that the fate of most eukaryotic cells is controlled by specific cell signaling pathways. Therefore, it is reasonable to assume that cell-signaling cascades may be very important for the development of Plasmodium. Systematic analysis of molecules involved in cell signaling events that occur during the course of P. falciparum development need to be pursued. A recently published genome sequence (1) and earlier studies suggest that several homologues of eukaryotic signaling proteins, such as protein kinases and phosphatases, are conserved in P. falciparum (2–13). The major challenge is to understand how these enzymes integrate in cellular machinery of Plasmodium and what roles they play in parasite development. The answer to these questions could lead to identification of important signaling pathways involved in the development of this parasite.

We are interested in understanding the role of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB) in P. falciparum. The PI3K-PKB-mediated signaling is important for proliferation and survival of several eukaryotic cell types such as fibroblasts and neuronal cells (14). After PI3K activation, 3′-phosphorylated phosphoinositides (phosphatidylinositol 3,4-diphosphate or phosphatidylinositol 3,4,5-trisphosphate) are generated, and they bind to the PH domain present at the N terminus of PKB, resulting in its translocation to the cell surface and phosphorylation-dependent activation (15–17). This regulatory phosphorylation is carried out by phosphatidylinositol-dependent kinase 1 (PDK1), which also regulates other members of the AGC group of protein kinases like PKA and isoforms of PKC (18, 19). However, maximal catalytic activity is achieved only upon phosphorylation of a C-terminal site. PKB targets a wide variety of cellular targets ranging from transcription factors, anti/pro-apoptotic proteins, enzymes involved in glycolysis metabolism, etc. (15–17). Function of neither PI3K nor PKB in Plasmodium or any other protozoan parasite is understood.

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The abbreviations used are: PI3K, phosphatidylinositol 3-kinase; PKA, cAMP-dependent protein kinase; PKB, protein kinase B; PKC, protein kinase C; PKG, cGMP-dependent kinase; PfPKB, Plasmodium falciparum homologue of protein kinase B; ΔPfPKB, catalytic domain of PfPKB with the C-terminal extension and without the N-terminal region; AGC kinases, a family of kinases belonging to the same class as protein kinase A, G, and C; BLAST, basic local alignment search tool; PH, Pleckstrin homology domain; PDK1, PI3-dependent kinase; GST, glutathione S-transferase; NTR, N-terminal region of PfPKB; PlasmoDB, sequence database of Plasmodium; MSP1, merozoite surface protein 1; ORF, open reading frame; contig, group of overlapping clones.

PKB refers to mammalian PKB in general. For the purpose of sequence comparison and amino acid number assignment human PKBα (accession number A46288) was used.

This paper is available on line at http://www.jbc.org
We report identification and biochemical characterization of a PKB homologue from *P. falciparum*, PFKPB. Although it shares significant homology with mammalian-PKB catalytic domain, PFKPB lacks a phosphoisoitride interaction domain (PH domain). Biochemical studies suggested that PFKPB auto-phosphorylation is pivotal for its activation, unlike the mammalian enzyme, which needs phosphorylation by PDK1. PFKPB was cloned at its highest levels during the schizont/merozoite stage of the parasite lifecycle. Studies using a pharmacological inhibitor suggested that PFKPB activity may be important for parasite development.

**EXPERIMENTAL PROCEDURES**

**P. falciparum Cultures**—*P. falciparum* 3D7 strain was cultured at 37 °C in RPMI 1640 medium using either AB− human serum or 10% Alumnum II (Invitrogen) as previously described (20). Cultures were gassed with 7% CO2, 5% O2, and 88% N2. Synchronization of the parasites in culture was achieved by sorbitol treatment (21).

**Molecular Cloning and Mutagenesis of PFKPB DNA**—Human PKBβ sequence was used to BLAST search (in tBLASTn mode) the Plasmodium genome sequence at Sanger Center, The Institute of Genomic Research, and Stanford University (chromosome 12). A contig highly homologous to PKB was found on chromosome 12. The longest open reading frame (ORF) spanning this region was named PFKPB (described in detail under “Results”). PCR was performed using primers (sequences given below) based on the PFKPB ORF (Fig. 1). Total RNA was isolated from asynchronous *P. falciparum* 3D7 cultures, and reverse transcription was performed using random hexamers provided in the Thermoscript reverse transcription-PCR kit (Invitrogen). Complementary or genomic DNA was used as the template in PCR reactions, which were performed using platinum Taq polymerase (Invitrogen) with the following cycling parameters: 94 °C for 2 min initial denaturation followed by 30 cycles at 94 °C for 30 s, 42 °C for 30 s, 72 °C for 2 min and final extension at 72 °C for 10 min. The following primer sets were used for cloning the full-length PFKPB gene (PFKPB_1 and PFKPB_2) and its catalytic domain, ΔPKB (PFKPB_2 and PFKPB_3) forward (PFKPB_1), 5'-ATGATCATATACATGTACCATATCTATGCCC-3'; reverse (PFKPB_3), 5'-TCTCATTTTGTACCTGTTTCTCTATAAGTTG-3'. PCR products were cloned in pGEM-T easy vector (Promega), and several clones were sequenced by automated DNA sequencing. All site-directed mutagenesis studies were performed using the QuikChange kit (Stratagene) following the manufacturer's instructions and using primers 5'-GAAAACATTTAACAAGCATTGACGGAAGATCC-3' and 5'-GGGGTTCGCAATGTTTTAGTTAATAGGTCTT-3' for mutant S271D, 5'-GATATATGAATACATGTACCATATCTATGCCC-3'; forward, (PfPKB_2), 5'-GAAACCATTTAACAAGCATTGACGGAAGATCC-3' and 5'-GATAGATCATATACATGTACCATATCTATGCCC-3'; reverse, (PfPKB_3), 5'-TCTCATTTTGTACCTGTTTCTCTATAAGTTG-3'. PCR products were cloned in pGEM-T easy vector (Promega), and several clones were sequenced by automated DNA sequencing.

**Expression and Purification of Recombinant PFKPB and Its Variants**—PFPKPB or ΔPFKPB was amplified using primers (described above) containing overhangs for BamHI and XhoI restriction enzymes to facilitate cloning into a GST fusion protein expression vector pGEX-4T1 containing overhangs for BamHI and XhoI restriction enzymes to facilitate cloning into a GST fusion protein expression vector pGEX-4T1. Cultures were grown in LB media containing kanamycin (25 μg/ml) or ampicillin (100 μg/ml) and chloramphenicol (50 μg/ml). Protein expression was induced by treatment of bacterial cultures with 1 mM IPTG-1-thio-β-D-galactopyranoside at 18 °C for 12 h. Recombinant His6 NTR protein was extracted in denaturating extraction buffer (50 mM Tris, pH 7.4, 300 mM NaCl, and 8 M urea), and the clarified lysate was incubated with nickel nitrotriocatic acid-agarose (Qagen) was washed at 4 °C, incubated overnight at room temperature. The resin was poured into a column, washed with extraction buffer, and eluted in extraction buffer containing 25–500 mM imidazole. Fractions containing the protein of interest were pooled and dialyzed against buffers containing decreasing amounts of urea (25 mM Tris, pH 7.4, 150 mM NaCl, 1 mM dithiothreitol, 0–6 M urea) to refold the recombinant protein. GST-NTR was purified as described above for other GST fusion proteins. Recombinant proteins were estimated by SDS-PAGE and analyzed by densitometry.

**Assay of PFKPB Activity**—GST fusion proteins of PFKPB (its deletions or its mutants) or 10 μl of PFKPB immunoprecipitated from parasite lysate (see below) were assayed for catalytic activity in a buffer containing 50 mM Tris, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, and 100 μM γ-32P-ATP (6000 Ci/mmol) using histone H1, histone H2A, or a small peptide substrate of PKB, “crosstide” (22), as phosphate-acceptor substrates. Typically, reactions were carried out for 20–60 min at 30 °C and were terminated by boiling the samples in SDS-PAGE sample buffer. Reaction mixtures were electrophoresed on 15% SDS-PAGE gel (for histone H2A) followed by autoradiography to visualize phosphate incorporation in histone H2A. In some experiments, GST-NTR (0.1 μg) and His6-NTR (0.1 μg) were preincubated with ΔPFKPB (0.4 μg) before the addition of substrate and ATP.

When peptide substrates were used, reactions were stopped by boiling the samples on SDS-PAGE gel (for histone H2A) followed by autoradiography to visualize phosphate incorporation in histone H2A. In some experiments, GST-NTR (0.1 μg) and His6-NTR (0.1 μg) were preincubated with ΔPFKPB (0.4 μg) before the addition of substrate and ATP.

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with Texas Red were used to localize merozoite surface protein 1 (MSP1) and PfPKB, respectively. Parasite nuclei were localized with Hoechst 33342. Stained parasites were visualized using either a Nikon TE2000 or a Zeiss confocal microscope. Images were analyzed and merged using Image Pro plus or Adobe Photoshop software.

Inhibition of PfPKB Activity and Parasite Growth by Go 6983 — Recombinant GST-H9004 PfPKB was preincubated for 15 min with varying concentrations of Go 6983 or Go 6976 (Biomol or Calbiochem) prepared in 0.1% Me2SO or with 0.1% Me2SO. The enzyme activity was assayed as described above. To monitor the effect of Go 6983 on \textit{P. falciparum} growth, parasites were plated at 1% parasitemia containing mostly rings and were treated either with the indicated concentrations of Go 6983 or 0.1% Me2SO. At the indicated time points, thin films were prepared and stained with Giemsa reagent, and the number of intra-erythrocytic parasites was counted by using microscopic examination. Parasites were counted from several different fields and also different experiments. The data (Fig. 8) are represented as the ratio of percent of parasite-infected erythrocytes in drug-treated cultures to the ones in control cultures, which were treated with 0.1% Me2SO.

RESULTS

Identification and Molecular Cloning of the PfPKB Gene — To identify a PKB homologue in \textit{P. falciparum}, human PKB sequence was used to search the \textit{P. falciparum} genome sequence \textit{in silico}. A match on chromosome 12 sequence database with high sequence homology to the catalytic domain of human PKB (71%) was found. A long and uninterrupted 1.34-kilobase (encoding a polypeptide of 446 amino acids) ORF encompassing this region was designated as PfPKB. PfPKB belongs to the AGC kinase family, which possesses a motif with consensus sequence (S/T)(F/L)CGTP(D/E)Y in its activation loop region (Fig. 1, A and B). Phosphorylation of S/T (at the first position in this sequence) is pivotal for their activation (19). In addition, PfPKB has a hydrophobic motif at its C-terminal end (Fig. 1) that is also present at the C-terminal end of several AGC kinases, although its sequence is only loosely conserved (23).

PfPKB ORF was amplified using either cDNA or genomic
P. falciparum Homologue of PKB

DNA as template, and the amplified PCR products appeared to be identical in size (data not shown). This suggested that PfPKB is at least 1.34 kb in size and introns are absent from this contig, which was confirmed by DNA sequencing. The deduced PfPKB sequence (Fig. 1A) shows that it has all kinase subdomains (26) and has a hydrophobic motif at the C terminus present in several AGC class kinases (23). It also has a putative signal sequence at its N-terminal end. Interestingly, PfPKB shares high sequence homology with catalytic domains of PKC isoforms α, β, γ, (−64%, Tables I and II). It should be noted that a PKC homologue has not yet been identified in the Plasmodium genome. Our in silico analysis suggested that PfPKB may be the closest relative of PKC in P. falciparum. Strikingly, PfPKB does not contain a PH domain usually found in PKB of higher eukaryotes. This domain is important for binding to phosphoinositides. During the process of screening for full-length PfPKB, we found clones containing intronic sequences that could result in different-size ORF. This could be a result of alternative splicing or defects in transcripts.

Recombinant PfPKB Catalytic Domain (ΔPfPKB) Is Active—To get insight into the catalytic mechanism of PfPKB, the catalytic domain of this enzyme with the C-terminal extension (ΔPfPKB, indicated in Fig. 1A) was expressed as a GST fusion protein in E. coli (Fig. 2A). Catalytic activity of recombinant ΔPfPKB was assayed using histone IIa of histone H1 as substrates of this enzyme. GST−ΔPfPKB phosphorylated histone IIa (Fig. 2B) and H1 (data not shown). It has been observed that AGC protein kinases (PKA, PKC, PKB, PKG) prefer basic residues in the vicinity of the target phosphorylation sites (30). Crostide, a peptide with sequence GRPRTSSEFG that has been shown to be a good substrate for PKB (22), was phosphorylated by ΔPfPKB (Km = 20 μM) (Fig. 2C). ΔPfPKB also phosphorylated a myelin basic protein-derived peptide (QKRPSQRSKYL) but with 2-fold less efficiency (data not shown here). Because the difference in these peptides was in the location of the basic residues corresponding to the phosphorylatable serine residues, it reflects the preference of PfPKB for basic residues at appropriate position in its substrates.

PfPKB May Be Regulated by Autophosphorylation of Ser-271 and Additional Phosphorylation of Ser-442—PfPKB is catalytically activated by phosphorylation of activation loop at Thr-309 by PDK1 (18, 23, 31), and deletion of the N-terminal PH domain of PKB results neither in its catalytic activation nor in autophosphorylation; it requires phosphorylation by PDK1 to be active (32). In contrast, catalytic activity of recombinant ΔPfPKB was observed in the absence of any exogenous kinase (Fig. 2, B and C). Instead, it exhibited autophosphorylation (Fig. 2D, lane 1). These data suggest that autophosphorylation may be responsible for regulating PfPKB catalytic activity.

Thr-309 phosphorylation in PKB results in conformational changes crucial for its catalysis (15, 23, 31). Because Ser-271 in PfPKB is complementary to Thr-309, it may be in a similar strategic location in the kinase catalytic core (Fig. 1B) and may play a role in PfPKB activation. To test this, Ser-271 of PfPKB was replaced by alanine, and the effect on its catalytic activity was assessed. Mutation of this serine to alanine resulted in almost a complete loss of ΔPfPKB activity as the S271A mutant failed to phosphorylate either histone (Fig. 3A) or crostide (Fig. 3B). Moreover, this mutation resulted in a concomitant loss in autophosphorylation of ΔPfPKB (Fig. 3C). It has been observed that replacement of Ser/Thr residues by negatively charged aspartate (Asp) or glutamate (Glu) could mimic their phosphorylated state. Replacement of S271A to S271D resulted in a significant recovery of ΔPfPKB activity, which was lost due to the S271A mutation (Fig. 3, A and B). Collectively, these data suggest that autophosphorylation of Ser-271 is a prerequisite for PfPKB activation. A ΔPfPKB mutant defective in Thr binding was generated by replacing a crucial lysine residue, which is conserved in all protein kinases, to methionine. It neither exhibited detectable autophosphorylation nor kinase activity, confirming that PfPKB autophosphorylation is responsible for its activity (data not shown).

PKB and other mammalian AGC family protein kinases share several other functional similarities such as dependence on additional phosphorylation of their C-terminal end to achieve maximal activity (17). This phosphorylation site (Ser-474 in PKB) is usually part of a loosely conserved hydrophobic motif with the sequence FXF/S/T/Y (23). PKA has a negatively charged residue (Asp or Glu) at this position, which complements the function of phosphorylated Ser or Thr (23). PfPKB contains a hydrophobic motif (YYEFSG) at its C terminus (Fig. 1). Ser-442 in this region was replaced by aspartic acid in addition to the above-described S271D mutation to mimic phosphorylation at this position. The double mutant (S271D, S442D) exhibited catalytic activity even higher than that of single mutant, S271D (Fig. 4). These data suggest that acidic environment provided by phosphorylation at this site may be important for maximal activation of PfPKB, an observation consistent with mammalian PKB.

NTR of PfPKB Negatively Regulates Its Activity—A BLAST search using the NTR amino acid sequence did not show any significant sequence homology with proteins in the nonredundant protein data base. To study the effect of N-terminal region (NTR) on the activity of PfPKB, full-length PfPKB (containing both the N-terminal region and the catalytic domain) was expressed, and its catalytic activity was compared with that of ΔPfPKB. PfPKB exhibited only marginal catalytic activity in comparison to its NTR deleted version, ΔPfPKB (Fig. 5, A and B). In addition, PfPKB also lacked detectable autophosphorylation activity (Fig. 5C). Collectively, these observations suggest that NTR may modulate PfPKB activity by preventing its autophosphorylation, thus preventing its catalytic activation. To determine if NTR directly causes PfPKB inhibition, it was expressed as a GST fusion or a His6-tagged protein. When recombinant NTR was incubated in a kinase assay mix with ΔPfPKB, its activity (Fig. 5D) as well as its autophosphoryla-

### Table I

| Plasmodium gene | Homology | Experimental identification |
|-----------------|----------|-----------------------------|
| Ch12 glm521     | 71%      | PfPKB (this work)           |
| Ch9 glm401      | 56%      | PKPKA (9, 13)               |
| Ch14 glm391     | 56%      | PKPPG (5)                   |

### Table II

| Kinase | Homology |
|--------|----------|
| PKB    | 71%      |
| PKCa−β−γ | 64%    |
| cAMP-dependent protein kinase | 58% |
| cGMP-dependent protein kinase | 53% |

**%** Homology represents the percent similarity in amino acids that are identical and belong to a similar chemical class. It is indicated as % similarity in the output of the BLAST program.
tion (Fig. 5E) was inhibited dramatically. Interaction of NTR with the catalytic region of PfPKB may either cause conformational changes unfavorable for catalysis and/or it may restrict entry of the peptide substrate in the catalytic cleft of the enzyme, resulting in its inability to perform efficient catalysis.

Because the data illustrated in Fig. 3 indicate that phosphorylation of Ser-271 (or its mutation to D) activates ΔIPPKB, it was worth testing the effect of S271D mutation on activation of full-length PfPKB. S271D mutant exhibited catalytic activity higher than that of PfPKB (Fig. 5F), suggesting that conformational changes due to phosphorylation-mimicking mutation of Ser-271 to Asp could result in an increase in activity of full-length PfPKB. Collectively, the results described above indicate that NTR may prevent the activation of PfPKB by inhibiting its ability to autophosphorylate.

**PfPKB Is Expressed in Schizonts and Merozoites**

Western blotting, immunofluorescence, and reverse transcription-PCR techniques were used to examine the expression pattern of PfPKB during *P. falciparum* blood stage development. For this purpose, polyclonal antisera were raised against a synthetic
peptide derived from the C terminus of PfPKB (Fig. 6A). PfPKB expression was predominantly observed in the schizont stages of the parasite as indicated by a band of ~52 kDa, which is consistent with the predicted molecular mass of 50 kDa (Fig. 6B). Reverse transcription-PCR analysis indicated that PfPKB transcripts were mainly present in schizont stages and were observed in the trophozoites at only very low levels (Fig. 6C). PfPKB immunoprecipitated from schizont lysates was able to phosphorylate crosstide, indicating PfPKB is active in the schizonts of P. falciparum (Fig. 6D).

Immunofluorescence studies revealed that PfPKB was present mainly in the mid-late schizont stages of the parasite; other mono-nucleated stages did not show any detectable PfPKB expression (Fig. 6E). To probe if PfPKB is localized at the cell surface, co-localization studies were performed using antisera against MSP1, a marker for merozoite surface. PfPKB exhibited a diffused staining pattern in segmented schizonts, indicating its presence in the cytoplasm, and also exhibited some co-localization with MSP1 (Fig. 6F). In free merozoites PfPKB seems to be localized at the apical end (Fig. 6G), a region that is important for erythrocyte invasion. PfPKB also co-localizes with EBA175, a micronemal protein (data not shown).

Go 6983 Is an Inhibitor of PfPKB Activity and Inhibits Parasite Growth—We were interested in identifying PfPKB inhibitors to help us understand the structure-function relationship of this Plasmodium protein kinase and, most importantly, its physiological role in promoting parasite growth and development in the host. Because there are no known or available inhibitors of mammalian PKB, we considered the possibility of inhibitors of other related kinases as putative candidates for PfPKB. For this purpose PKC inhibitors were selected based on following reasons. 1) In silico analysis suggested that catalytic domain of PfPKB was most closely related to PKC in comparison to other AGC kinases (Table II); 2) it appears that P. falciparum does not have a PKC homologue. Go 6983 and Go 6976 (Fig. 7A) are isoform-specific PKC inhibitors that target the ATP binding site (33). Inhibition of any other AGC kinases by these compounds has not yet been reported. The effect of these compounds on in vitro recombinant PfPKB activity was tested. Go 6983 inhibited PfPKB activity, as judged by its ability to phosphorylate histone II aS (Fig. 7B) and crosstide (Fig. 7C), with an IC_{50} of ~1 μM. In contrast, Go 6976 was unable to inhibit the PfPKB activity even at 10 μM concentration (Fig. 7D). These data suggested that Go 6983 could be a useful tool for manipulating PfPKB activity in P. falciparum for deciphering its role in the life cycle of the parasite. To test this, synchronized P. falciparum cultures were incubated with Go 6983, and parasite growth was monitored at different times.
There were no apparent effects of this drug until parasites reached the late schizont/segmenter stage (40–44 h). A significant decrease in parasitemia was observed in drug-treated cells subsequent to this point. In Go 6983-treated cells, the number of rings in the following cycle was markedly less compared with the control cultures (Fig. 8A). This observation correlates well with the expression profile of PfPKB in the schizont/merozoite stages of the life cycle (Fig. 6). It is reasonable to state that the major effects of Go 6983 were due to its interaction with *Plasmodium* cellular machinery and not a
result of effects on erythrocytes, since other parasite stages and erythrocytes appeared to have normal morphology.

To further establish the effect of this inhibitor, *P. falciparum* schizonts were incubated with Go 6983, and development of the parasite was monitored. Although untreated cells formed rings within $4 \sim 6$ h, Go 6983 treatment resulted in an almost 60% decrease in formation of new rings (Fig. 8B). These data suggest that this drug targets directly the late schizont stages or the merozoites, which would prevent invasion of erythrocytes and subsequent formation of fresh rings.

**DISCUSSION**

Role of signal transduction events in the development of most protozoan parasites is unclear. Especially, information about molecular machinery involved in carrying out these events is lacking. One of our interests is to understand the role of the classical PI3K, PKB pathways in *P. falciparum*. We have identified a PI3K homologue and a PKB homologue (reported here) in *P. falciparum* that suggests that signaling pathways regulated by PI3K and PKB are likely to be conserved in this parasite, although they may not necessarily operate in a manner similar to higher eukaryotes (see the discussion below).

PIPKB shows significant sequence homology to the catalytic domain of protein kinase B in higher eukaryotes. In addition it also shares almost 65% sequence homology with the catalytic domain of protein kinase C, another AGC class Ser/Thr kinase. So far, a PKC homologue has not been identified in *P. falciparum*. Our analysis suggested that PIPKB is closest to, besides PKB, mammalian PKCa, β, γ at the sequence level. PIPKB does not have a PH domain, which plays an important role in subcellular localization and activation of PKB by binding to

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3 P. Sharma, manuscript in preparation.
3'-phosphorylated phosphoinositides, a product of PI3K in mammalian cells (16, 17). It is interesting to note that yeast and T. cruzi homologues also do not have a PH domain (27–29), suggesting that PKB in these unicellular eukaryotes may not be directly regulated by phosphoinositides. The N-terminal region of P.fPKB does not share any significant homology to proteins in various databases. It inhibits PPKB catalytic activity by preventing its autophosphorylation. Often, regulatory domains are found at the N or C terminus of protein kinases, and binding of effector molecules (e.g. calmodulin in interaction with calmodulin-dependent kinases, diacylglycerol, and calcium; in the case of PRC, interaction of phosphoinositides with PKB) to these domains brings about conformational changes that result in modulation of their catalytic activity. There is a possibility that interaction with effector molecules may result in PPKB activation. Identification of these effectors could provide important clues about its cellular regulation.

In contrast to mammalian PKB that is activated by PDK1, PPKB appears to be activated by autophosphorylation of Ser-271. This serine residue is complementary to Thr-309 of PKB, and phosphorylation of this site by PKB is crucial for PKB activation. Despite sequence conservation in the vicinity of Ser-271, human PDK1 was unable to activate PPKB (data not shown). Moreover, a PDK1-like enzyme is absent in Plasmodium, suggesting that autophosphorylation is the likely mode of activation of PPKB. Several AGC kinases (e.g. conventional PKC isoforms) are regulated by autophosphorylation and do not appear to require PDK1 (34). It is interesting to note that PKB homologue from another protozan parasite, T. cruzi, is also regulated by autophosphorylation (27).

It has been shown that the N-terminal PH domain-deleted versions of PKB do exhibit any significant catalytic activity or autophosphorylation and its activation is dependent on PDK1-mediated phosphorylation (32). Several AGC kinases possess a loosely conserved hydrophobic patch of acidic residues at their C-terminal end. A recent crystal structure of PKB revealed that phosphorylation of Ser-474 in this region promotes conformational stability of the N-lobe of the kinase, resulting in a conformation favorable for catalysis (23, 31). Enhancement of PPKB activity upon mutation of Ser-442 to Asp suggests that a conformation favorable for catalysis (23, 31).

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