Malaria, caused by infection with intracellular protozoan parasites of the genus *Plasmodium*, is responsible for 300 million to 600 million clinical cases annually (49), resulting in the deaths of up to 3 million people every year (9, 10). The need for novel intervention strategies is rendered more acute by the increasing evidence showing that CK2 is an attractive target for antineoplastic and antiviral drugs (46). The parasite life cycle is complex, with a succession of proliferation and differentiation events, in the regulation of which protein phosphorylation plays a crucial role (34). Consistent with its multiple substrates, the enzyme plays a crucial role in many cellular processes, including differentiation, proliferation, and translation. The mammalian holoenzyme consists of two catalytic alpha or alpha’ subunits and two regulatory beta subunits. We report the identification and characterization of a *Plasmodium falciparum* CK2 alpha orthologue, PfCK2alpha, and two PfCK2beta orthologues, PfCK2beta1 and PfCK2beta2. Recombinant PfCK2alpha possesses protein kinase activity, exhibits similar substrate and cosubstrate preferences to those of CK2alpha subunits from other organisms, and interacts with both of the PfCK2beta subunits in vitro. Gene disruption experiments show that the presence of PfCK2alpha is crucial to asexual blood stage parasites and thereby validate the enzyme as a possible drug target. PfCK2alpha is amenable to inhibitor screening, and we report differential susceptibility between the human and *P. falciparum* CK2alpha enzymes to a small molecule inhibitor. Taken together, our data identify PfCK2alpha as a potential target for antimalarial chemotherapeutic intervention.

### MATERIALS AND METHODS

Expression and purification of the three PfCK2 subunits. Oligonucleotides were designed to amplify the PfCK2alpha open reading frame (ORF) from *P. falciparum* (clone 3D7A) cDNA. The forward (5’-GGGGAGATCTATGGAAAATAGTGATTCGAATAAAGAC-3’ (5’-GGGGAGATCTATGGAAAATAGTGATTCGAATAAAGAC-3’)) and reverse (5’-GGGGAGATCTTCTTCTTCC-3’ (5’-GGGGAGATCTTCTTCTTCC-3’)) primers carried BamHI and SalI sites, respectively (underlined). Oligonucleotides were also designed to amplify the PfCK2beta1 and PfCK2beta2 ORFs from *P. falciparum* (clone 3D7A) cDNA. The PfCK2beta1 forward (5’-GGGGAGATCTTCTTCTTCC-3’) and reverse (5’-GGGGAGATCTTCTTCTTCC-3’) primers carried BglII and SalI sites, respectively (underlined). The PfCK2beta2
sequence has a long N-terminal extension. Oligonucleotides were designed to amplify the Pick2β2 sequence, lacking the N-terminal extension, from _P. falciparum_ (clone 3DA7) cDNA. The forward (5′-GGGGGTACCATGGAGAC AACAGTGTCTGGATTGTTG-3′) and reverse (5′-GGGGCTCACTACTTTGACACTTCAAGTGGAGATTCGCCG-3′) primers carried BamHI and Sall sites, respectively (underlined). The short version of Pick2β2, lacking the N-terminal extension, was named shPick2β2. Catabolically inactive (“kinase dead”) Pick2Δ was obtained by site-directed mutagenesis (K22M) of pGEX-Pick2β2 by overlap-extension PCR (23). All cloning primers are provided in Table S1 in the supplemental material. All PCR products were verified by sequencing in the vector pGEM-T Easy (Promega) and then subcloned into the plasmid pGEX-4T3 (GE Healthcare) to generate _N-terminal glutathione S-transferase_ (GST) fusions. The shPick2β2 sequence was also inserted between the BamHI and NotI sites of the plasmid pQE-30 for expression with an N-terminal His tag. A pET29 vector containing _P. falciparum_ CKε2 in frame with a C-terminal His tag sequence was a kind gift from D. Chakrabarti. The pGEX-4T3 constructs were expressed in _Escherichia coli_ BL21 Gold cells, SG13009 cells were used for expression from the pQE30 plasmid, and the pET29 construct was expressed in _E. coli_ BL21(DE3) cells for 2 h at 20°C with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), GST-tagged proteins were purified on glutathione-agarose beads (Sigma), and His-tagged proteins were purified on Ni2+-agarose beads (Qiagen), following the manufacturer’s recommendations.

**Construction of the knockout (KO) plasmid pCAM-BSD-KOPick2α.** A fragment from the Pick2α ORF was amplified by PCR and inserted between the BamHI and NotI sites of the pCAM-BSD plasmid (47), which contains the _Aspergillus terreus_ triticidin-β-deaminase gene, whose gene product confers resistance to the drug tricitidin. Cloning primers are listed in Table S1 in the supplemental material.

**Construction of the complementation plasmid pCHD-Pick2α.** A plasmid for in vivo transcriptional expression of Pick2α subunits was constructed as follows: the full-length Pick2α coding sequence was first inserted between the BglII and NotI sites of the plasmid pHGB (51) and then transferred into the plasmid pCHD-1/2 (51) by a Gateway LR ligation reaction according to the manufacturer’s instructions (Invitrogen). The plasmid pCHD-1/2 includes a cassette encoding human dihydrofolate reductase, conferring resistance to the antifolate drug methotrexate and a kanamycin cassette for _E. coli_.

**Kinase assays.** Standard kinase reactions (30 μl) occurred in kinase buffer (20 mM Tris-HCl [pH 7.5], 20 mM MgCl2, 2 mM MnCl2, and 10 μM ATP) containing 0.037 MBq [32P]ATP (370 MBq/ml; GE Healthcare), 1 μM unlabeled ATP/GTP and diluted serially to ensure a constant ratio of labeled- to-unlabeled ATP/GTP. The NEB peptide RRADDSSDD (100 μM) was used as the substrate. Reaction mixtures contained 36 ng of Pick2α alone or in combination with 36 ng of Pick2β1/shPick2β2.

**Measurement of 50% inhibitory concentrations (IC50).** To test the effect of small molecule inhibitors on Pick2α, kinase activity was measured in the presence of increasing concentrations of these molecules. Stocks of the molecules contained dimethyl sulfoxide or ethanol as a solvent, and negative controls for the reactions were provided by reaction mixtures containing ethanol or dimethyl sulfoxide without the small molecule inhibitor. Kinase reactions were performed by the phosphocellulose method as detailed above.

**Interaction assay.** A mixture of 5 μg of each recombinant protein was incubated at 4°C for 30 min in 20 mM Tris-HCl (pH 7.5), 0.2 M NaCl, 0.1% Nonidet P40 (IGEPAL), and 10% glycerol. Glutathione-agarose beads were added to each reaction mixture. The tubes were rotated at 4°C for 1 h; the beads were recovered by centrifugation and washed four times in reaction buffer. Laemmli buffer was added to the beads, which were then heated to 100°C. Samples were separated by SDS-PAGE on 12% acrylamide gels and either stained or transferred to the membrane for Western blot analysis.

**Western blot analysis.** Western blotting was performed according to conventional protocols. Briefly, samples were separated by SDS-PAGE on a 12% acrylamide gel and blotted onto nitrocellulose. The membranes were blocked according to standard methods and incubated with rabbit anti-His antibody (1:1000; Santa Cruz Biotechnology) or subunit-specific antibodies generated in rabbits by BioGenes (Germany) against the Pick2α-derived peptide ADVNIH KPKKEYDY. A goat anti-rabbit secondary antibody coupled to horseradish peroxidase was used at a ratio of 1:10,000. Antibody was visualized using the enhanced chemiluminescence system (PerkinElmer).

**Parasite culture and transfection.** Cultures of the _P. falciparum_ strain 3DA (54) were maintained at 37°C in RPMI 1640 medium (Gibco) supplemented with 25 mM sodium bicarbonate, 2 mM glutamine, 300 mM hypoxanthine, 10 μg/ml gentamicin, and AlbuMAX II (Sigma). Cultures were seeded with 5% hematocrit and maintained at a parasitemia of 1 to 10% with daily changes of medium. The incubator was flushed with a gas mixture containing 5% CO2.

For transfection, asexual blood stage parasites were synchronized by sorbitol treatment (26) to obtain a majority of ring stage parasites. Forty-eight hours later, ring stage parasites were transfected by electroporation with 100 μg of purified plasmid DNA in Cytomix buffer as described previously (16, 17, 47). Blasticidin (2.5 μg/ml) was added to the culture medium to select for transformed parasites. Parasites under double selection had WR99210 (5 mM) added to the culture medium in addition to the blasticidin. Parasites were maintained in this supplemented medium from 2 days posttransfection.

**Preparation of parasite protein extract.** Parasite cultures were lysed in 0.15% saponin. After centrifugation and washing, the parasite pellets were sonicated in RIPA buffer (30 mM Tris, pH 8.0, 150 mM NaCl, 20 mM MgCl2, 1 mM EDTA, 0.5% Triton X-100, 1% Nonidet P40, 10 mM β-glycerophosphate, 10 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 10 mM benzamidine hydrochloride hydrate, and Roche complete cocktail protease inhibitors). The lysates were cleared by centrifugation (15,000 rpm for 15 min at 4°C), and the total amount of proteins in the supernatant was measured using the Bradford assay (8).

**DNA extraction and Southern blotting.** Parasite cultures were lysed in 0.5% saponin. The parasite pellets were resuspended in cold phosphate-buffered saline and treated with proteinase K (150 μg/ml) and 2% SDS at 55°C for 2 h. The genomic DNA was extracted in phenol/chloroform/isooamyl alcohol (25:24:1) and precipitated in ethanol and 0.3 M sodium acetate. The DNA was digested with HindIII.

**Primers used in genotype characterization.** For the detection of integration of the plasmid pCAM-BSD-KOPick2α into genomic DNA, the following primers were used (see Fig. 6A for numbering and see Table S1 in the supplemental material for the sequences): primer 1, CK2a3primeF; primer 2a, CK2aR; primer 2b, CK2a3primeR; primer 3, pCAM-BSDF; and primer 4, pCAM-BSDR.

For the detection of integration of the plasmid pCAM-BSD-HA, the following primers were used (see Fig. 7D for numbering and see Table S1 in the supplemental material for the sequences): primer 1, CK2aF; primer 2, CK2a3primeR; primer 3, pCAM-BSDF; and primer 4, pCAM-BSDR.

**RESULTS**

**Bioinformatics.** Phylogenetic analysis of _P. falciparum_ protein kinases identified the PlasmoDB (http://plasmodb.org/plasmo) sequence PF11_00996 as that of a _CKα_ orthologue (3, 57), with 65% amino acid sequence identity to _Homo sapiens_ CKα2. PF11_00996 was therefore named Pick2α. An alignment of Pick2α with _CKα_ subunits from _H. sapiens_ and _Zea mays_ (see Fig. S1 in the supplemental material) reveals that
PfCK2 possesses all 11 of the subdomains conserved across eukaryotic protein kinases (21, 22) and the majority of the conserved features of CK2 subunits (2). Just downstream from subdomain II is a putative nuclear localization signal, Pro-Val-Lys-Lys-Lys-Lys-Ile, conserved across CK2 homologues. PfCK2 also possesses three invariant residues common to CK2 family members; the ATP binding motif present in most other protein kinases is Gly-X-Gly-X-X-Gly, whereas in the CK2 family the motif is Gly-X-Gly-X-X-Ser (PfCK2, Gly50-Ser55). The most highly conserved amino acid motif specific to members of the CK2 family is Asp179-Trp-Gly181 (notation from PfCK2α; most protein kinases display Asp-Phe-Gly at this position). Likewise, Gly203-Pro-Glu205 (notation from PfCK2α) is a common feature of the family, which diverges from the Ala-Pro-Glu motif present in the vast majority of other protein kinases; thus, all three CK2-specific motifs are present in PfCK2α.

Two putative CK2β subunits were identified in P. falciparum (57), hereafter referred to as PfCK2β1 and PfCK2β2 (PlasmoDB identifiers PF11_0048 and PF13_0232, respectively). BLASTP searches using the putative PfCK2β1/PfCK2β2 amino acid sequences as queries confirmed their identities as CK2β homologues. An alignment with the human CK2β sequence (HsCK2β) (Fig. 1) reveals that many of the conserved features of CK2β subunits, including the four cysteine residues responsible for zinc finger formation (12), are present in PfCK2β1 and PfCK2β2 (e.g., Cys117, -122, -145, and -148 for PfCK2β1) (Fig. 1). The human CK2β sequence has a well-documented CK2 phosphorylation site at the N terminus (SSEE). PfCK2β2 possesses several phosphorylatable residues in the N-terminal region that are surrounded by a number of acidic residues, which could therefore be phosphorylated by CK2, and a TESSEE sequence at the C terminus reminiscent of the HsCK2β N-terminal phosphorylation site (MSSEE). The stretch of amino acids found to be necessary for the export of CK2 as an ectonkinase (CK2β amino acids E20 to K33) is largely conserved in the PfCK2β sequences, leading to the intriguing possibility that PfCK2 may be exported from the parasite. The acidic stretch responsible for downregulation of CK2 activity and association with the plasma membrane (HsCKβ amino acids D55 to D64) (29, 32) is present in PfCK2β1 (D68 to D75) and extended in PfCK2β2 (D207 to E226). This insertion occurs in a region looping out from the main protein structure (12) and is not unique; for example, Saccharomyces cerevisiae CK2β has an insertion sequence of 30 amino acids in this location. Along with the insertion region, PfCK2β2 has a highly acidic and repetitive N-terminal exten-
sion that is not found in other CK2β subunits. The N-terminal
region is at the periphery of the 3D structure of the human
CK2β peptide (12, 35) and is not a conserved part of the CK2β
structure and thus may possibly function as a docking site or
region that interacts with binding partners or substrates of the
PfCK2 holoenzyme. The human CK2β is phosphorylated at
S209 in a cell cycle-dependent manner by p34cdc2 (19, 30, 33),
although the function of this phosphorylation is unknown.
Both PfCK2β subunits possess serine residues near the C ter-
minus that could be phosphorylated.

Microarray data available on PlasmoDB reveal that the
mRNAs encoding all three subunits are detectable throughout
the parasite life cycle (7, 28). Expression of the PfCK2α pro-
tein in asexual blood stage parasites was verified by Western
blot analysis (see Fig. 7F).

In vitro activity of PfCK2α. Kinase activity of bacterially
expressed recombinant GST-PfCK2α was detected by an in
vitro kinase assay (Fig. 2, top). GST-PfCK2α autophosphory-
lates (the 66-kDa band present in each lane in Fig. 2 corre-
sponds to the size of the GST-tagged PfCK2α subunit) and is
capable of phosphorylating a range of exogenous substrates,
with strongest activity toward the caseins (Fig. 2, lanes 2 to 4)
and recombinant GST-tagged shPfCK2β2, a short version of
the PfCK2β2 subunit lacking the N-terminal extension (see
Materials and Methods) (Fig. 2, lane 7). There was no activity
against the GST moiety alone (Fig. 2, lane 8), indicating that
the activity in lane 7 was against the beta subunit itself and that
the autophosphorylation is against the PfCK2α subunit itself.
We did not detect any activity against the PfCK2β1 subunit
(data not shown). These observations are consistent with gen-
eral preferences of CK2 homologues for substrates with highly
acidic phosphoacceptor sites (11, 25, 39, 52, 56); several po-
tential such sites are present on PfCK2α. Parallel assays per-
formed with an inactive PfCK2α mutant (Lys72Met; the Lys
residue is required for correct orientation of the ATP mole-
cule) were negative for kinase activity, confirming that activity
is indeed due to PfCK2α (Fig. 2, bottom). PfCK2α autophos-
phorylates by a transreaction (Fig. 2B): GST-PfCK2α and
PfCK2α-His autophosphorylate (Fig. 2, lanes 1 and 3), while
GST-K72MPfCK2α does not (Fig. 2, lane 2) but is phosphor-
ylated in the presence of PfCK2α-His, indicating that at least a
proportion of the autophosphorylation of PfCK2α occurs by an
intermolecular reaction.

PfCK2α shares features in common with CK2α from other
systems. A feature often cited as being characteristic of CK2
enzymes is that they have similar affinities for GTP and ATP.
PfCK2α has a $K_m$ of 16.7 μM and $V_{max}$ of 6.6 nmol/min for

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**FIG. 3.** PfCK2α kinetics. The enzyme kinetics of PfCK2α in a Line-
weaver-Burke presentation. The experiments were performed in triplic-
ate, the data points represent the means, and the error bars repre-
sent three standard deviations. (A) The graph was obtained by linear
regression of the enzyme kinetic data for ATP and GTP. The inter-
cepts on the $x$ axis give the negative reciprocal of the $K_m$, and the inter-
cepts on the $y$ axis give the reciprocal of the $V_{max}$. (B) The graph
was obtained by linear regression of the enzyme kinetic data for the
NEB peptide RRRADDSDDDDD. The intercept on the $x$ axis gives the
negative reciprocal of the $K_m$.

**FIG. 4.** PfCK2α and HsCK2β interact in vitro. To further test the
interactions of the alpha and beta subunits, two substrates that are
phosphorylated by the CK2 holoenzyme and not by the CK2α subunit
alone were included in kinase assays with mixtures of human and P.
falciparum alpha and beta subunits. (A) Phosphorylation of the
c1F3β[1-22] peptide (40, 45) by PfCK2α-His or HsCK2α in the pres-
ence and absence of GST-PfCK2β1, GST-shPfCK2β2, or HsCK2β was
measured by kinase assays, and the amount of radiolabel incorpo-
rated into the peptide was counted by scintillation. Results are shown as
the means of two experiments, with the error bars representing the stan-
dard deviations. (B) Phosphorylation of the GST-Olig2[1-177] protein
(27) by PfCK2α-His (lanes 1 to 4) or HsCK2α (lanes 5 to 8) alone
(lanes 1 and 5) or in the presence of GST-PfCK2β1 (lanes 2 and 6),
GST-shPfCK2β2 (lanes 3 and 7), or HsCK2β (lanes 4 and 8). Top,
autoradiogram; bottom, corresponding Coomassie blue-stained gel of
the kinase assay.
ATP and a $K_m$ of 34.9 $\mu$M and $V_{\text{max}}$ of 2.1 nmol/min for GTP (Fig. 3A). The enzyme displays a number of other features that confirm it as a true member of the CK2α family: (i) PfCK2α is able to phosphorylate the CK2 substrate peptide RRRADDSDDDD (NEB), with a $K_m$ of 137.5 $\mu$M (Fig. 3B); (ii) CK2α enzymes are known to have a wide variety of substrates (34), and correspondingly, PfCK2α (but not the K72M mutant protein used as a negative control) phosphorylates a number of proteins within heat-inactivated parasite protein extract (data not shown); (iii) the activity of PfCK2α is inhibited by the well-established CK2-specific inhibitor TBB (3,4,5,6-tetrabromobenzotriazole), with a similar IC50 curve to that of human CK2α (see Fig. 8C); and (iv) it can be recruited by the human CK2β subunit to phosphorylate the eIF2α-derived peptide (40, 45) and the Olig2 protein (27) (Fig. 4). This is in line with the established ability of human CK2, but not CK2α alone, to phosphorylate the substrates used in this experiment (27, 40).

**PfCK2α and the PfCK2 beta subunits interact in vitro.** To assess whether the two regulatory PfCK2 subunits are able to associate with PfCK2α in vitro, all three subunits were expressed in *E. coli* as His- or GST-tagged proteins and used in pull-down experiments. Mixtures of His- and GST-tagged proteins were prepared, from which proteins were pulled down using glutathione beads. The pulled-down proteins were then subjected to Western blot analysis using an anti-His antibody to detect any bound His-tagged protein that was copurified with the GST-tagged proteins. PfCK2α-His was copurified with both GST-tagged beta subunits but not with GST alone (Fig. 5A). The interaction does not significantly alter the $K_m$ for ATP (Fig. 5B) or the phosphorylation of calmodulin or the Olig2 protein (27) (Fig. 4). This is in line with the established ability of human CK2, but not CK2α alone, to phosphorylate the substrates used in this experiment (27, 40).

**PfCK2α is essential for completion of the erythrocytic asexual cycle.** We next wanted to determine whether PfCK2α plays essential functions in parasite survival. To generate a plasmid able to disrupt the PfCK2α gene, an internal fragment of the coding sequence, excluding the critical motifs Gly-X-Gly-X-X-Gly-Pro-Glu (subdomain VIII, required for structural stability of the C-terminal lobe) (see Fig. S1 in the supplemental material), was amplified and cloned into the transfection vector pCAM-BSD (47), which confers resistance to blasticidin. Integration of this construct (pCAM-BSD-KOPfCK2α) into the genomic locus by single-crossover homologous recombination (47) results in a pseudodiploid configuration, where both truncated copies will be unable to express a functional enzyme, since both will lack one of the essential motifs (Fig. 6A).

After two independent transfections of pCAM-BSD-KOPfCK2α into 3D7 parasites, integration was monitored in the blasticidin-resistant populations by PCR (Fig. 6B), using...
primer combinations that allow discrimination between the episome, the wild-type locus, and the disrupted locus. Only the episome and the wild-type locus were detectable, with no sign of integration even after prolonged culturing (16 weeks); in contrast, we regularly observe disruption of nonessential genes 6 to 7 weeks posttransfection (for an example, see reference 42). This might be due either to the fact that the presence of an intact PfCK2α gene is crucial for parasite asexual multiplication or to the possible nonrecombinogenicity of the locus. To verify that the PfCK2α locus is indeed recombinogenic, we proceeded to transfet wild-type parasites with a “3’-tagging” construct whose integration was expected not to cause loss of function of the target protein. We readily observed integration of the tagging construct (Fig. 7A to E) and size increase in the PfCK2α protein caused by the HA tag (1.1 kDa) (Fig. 7F). This demonstrates that the locus is accessible to recombination if no loss of function is incurred, as is presumably the case with HA tagging, and therefore strengthens the case that PfCK2α is essential for the parasite’s asexual cycle.

We nevertheless wanted to ascertain that PfCK2α can be disrupted if the enzyme is provided through expression of an extraneous copy of the gene. To this effect, a complementation plasmid was constructed, containing the full-length PfCK2α coding region under the control of the P. falciparum hsp86 promoter and preceding a 3’ untranslated region (namely, the Plasmodium berghei dihydrofolate reductase terminator sequence). The P. falciparum hsp86 gene (PF07_0029) displays a similar mRNA expression profile to the PfCK2α gene (28); therefore, its promoter is presumably appropriate to drive expression of the complementing protein. In parallel with the transfection of the pCAM-BSD-KOPfCK2α plasmid alone, further populations of parasites were cotransfected with both pCAM-BSD-KOPfCK2α and the complementation plasmid. PCR analysis (Fig. 6B, right) showed that disruption of the targeted locus occurred only in the doubly transfected, doubly resistant parasites. Southern blot analysis independently confirmed that integration occurred only in the doubly transfected parasites (Fig. 6C). The 13-kb band that represents the wild-type locus dramatically decreased in the doubly transfected parasites and was undetectable in two clonal lines (E7 and G9) that were derived from this culture by limiting dilution. There are multiple possibilities for the recombination of the KO and complementation plasmids with each other before or after integration, which could account for the additional bands of unexpected size observed (6 kb and 14 kb). The most important observation is that the wild-type band disappears only in the doubly transfected parasites.

Taken together, these data provide strong evidence that PfCK2α is essential to viability of the asexual erythrocytic stage parasites.

PfCK2α kinase activity is amenable to inhibition. Using a kinase-directed inhibitor library, we conducted a screen for compounds that inhibit PfCK2α. This screen identified the compounds Rottlerin and ML-7 as inhibitors of PfCK2α. The IC50 of these compounds were determined for both PfCK2α and HsCK2α (Fig. 8). While ML-7 inhibits both enzymes with an IC50 of roughly 3 to 4 μM, Rottlerin exhibits differential effects on the orthologues, inhibiting PfCK2α with an IC50 of 7 μM and HsCK2α with an IC50 of 20 μM. This result indicates that differential inhibition is possible, despite the high percent identity (65%) between the CK2α amino acid sequences of P. falciparum and Homo sapiens.

DISCUSSION

We have characterized a P. falciparum CK2α orthologue and confirmed that the recombinant enzyme exhibits kinase activity in vitro and exhibits features in common with other CK2α enzymes. PfCK2α contains the major motifs conserved across
CK2 catalytic subunits, phosphorylates acidic sequences, interacts with the putative PfCK2α subunits and the HsCK2β subunit, is inhibited by the classic CK2 inhibitor TBB (3,4,5,6-tetrabromobenzotriazole), with a similar IC₅₀ to that of HsCK2β, and is able to utilize GTP or ATP as a cosubstrate. We have also confirmed the identity of two PfCK2α subunits.

The N-terminal extension of PfCK2α is unusually long for CK2/α proteins, with 160 amino acids before the first conserved residue (Trp161 in PfCK2α). Most CK2/α subunits from vertebrates have only eight amino acids prior to this conserved residue (Homo sapiens, Gallus gallus, Mus musculus, Xenopus tropicalis, Bos taurus, and Danio rerio); this N-terminal extension is expanded in yeast (Saccharomyces cerevisiae, 37 residues), trypanosomatids (Trypanosoma brucei, 27 residues; Leishmania major, 21 residues), plants (Arabidopsis thaliana, 100 residues; Oryza sativa, 92 residues), and alveolates (Cryptosporidium parvum, 27 residues; Theileria parva, 34 residues). Within the alveolates, Plasmodium yoelii yoelii (125 residues) and Plasmodium vivax (157 residues) also have long extensions, but the extension of P. falciparum is the longest known. Homorepeat-containing proteins make up 35.7% of the proteome of P. falciparum, although the majority of these homorepeats are asparagines and lysines (48), unlike the polymers of acidic residues present in PfCK2α. One hypothesis for the function of this extension is the downregulation of the alpha subunit. Polyglutamate is a potent CK2 inhibitor (50), and the N-terminal extension of PfCK2α is rich in polyglutamate and polyglutamate. This beta subunit also possesses an insertion of extra acidic residues (including a stretch of 11 consecutive aspartates) (Fig. 1) in the acidic domain known to downregulate CK2 (32). We have not been able to purify PfCK2β with the N-terminal extension, and therefore this hypothesis remains to be tested. However, we showed that the presence of either beta subunit reduces the activity of PICK2α toward
FIG. 8. PfCK2α can be distinguished from HsCK2α by small molecule inhibitors. Two small molecules, ML-7 and Rottlerin, were identified in a primary screen as inhibiting the activity of PfCK2α to below 10% of the uninhibited enzyme. The inhibitors Rottlerin and ML-7 were included in increasing concentrations in kinase assays with 25 μM ATP, 36 ng of enzyme, and the peptide RRREDEESDDEE as substrate. Activity was measured using the phosphocellulose assay method, and results were scored as a percentage of the control (no inhibitor). (A) ML-7. (B) Rottlerin. Mean values from two experiments are shown, with the error bars representing the standard deviations. (C) The classical CK2 inhibitor, TBB, has a similar inhibitory profile for PfCK2α and HsCK2α.

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