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The Plasmodial Surface Anion Channel Is Functionally Conserved in Divergent Malaria Parasites

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The plasmodial surface anion channel (PSAC), a novel ion channel induced on human erythrocytes infected with Plasmodium falciparum, mediates increased permeability to nutrients and presumably supports intracellular parasite growth. Isotope flux studies indicate that other malaria parasites also increase the permeability of their host erythrocytes, but the precise mechanisms are unknown. Channels similar to PSAC or alternative mechanisms, such as the upregulation of endogenous host transporters, might fulfill parasite nutrient demands. Here we evaluated these possibilities with rhesus monkey erythrocytes infected with Plasmodium knowlesi, a parasite phylogenetically distant from P. falciparum. Tracer flux and osmotic fragility studies revealed dramatically increased permeabilities paralleling changes seen after P. falciparum infection. Patch-clamp of P. knowlesi-infected rhesus erythrocytes revealed an anion channel with striking similarities to PSAC: its conductance, voltage-dependent gating, pharmacology, selectivity, and copy number per infected cell were nearly identical. Our findings implicate a family of unusual anion channels highly conserved on erythrocytes infected with various malaria parasites. Together with PSAC’s exposed location on the host cell surface and its central role in transport changes after infection, this conservation supports development of antimalarial drugs against the PSAC family.

Red blood cells (RBCs) infected with Plasmodium falciparum, the most virulent cause of human malaria, exhibit markedly increased permeabilities to a diverse collection of solutes, including anions, sugars, amino acids, purines, some vitamins, and organic cations, all via the plasmodial surface anion channel (PSAC) (8). PSAC is induced some hours after parasite invasion and eventually reaches a functional copy number of between 1,000/cell and 2,000/cell, as estimated from comparisons of single-molecule and whole-cell patch-clamp measurements. Several functional properties suggest it is a novel ion channel. First, its rank order selectivity for anions (SCN⁻ > I⁻ > Br⁻ > Cl⁻) differs from nearly all human chloride channels and corresponds to the theoretical weakest binding site, Eisenman series I for anions (40). Another surprising selectivity feature is PSAC’s ability to exclude Na⁺ by more than 100,000-fold relative to Cl⁻ despite broad permeability to organic solutes of either net positive or negative charge (5).

Second, although infected RBCs are not excitable, PSAC exhibits voltage-dependent gating with significantly more frequent opening events at negative membrane potentials than at positive membrane potentials. This voltage dependence is also apparent in whole-cell recordings, in which current-voltage profiles exhibit “inward rectification.” Thus, it has become an important marker of PSAC. An important debate has evolved around this phenotype because some groups report mixed voltage dependences that suggest that ion channels other than PSAC might also be induced by the parasite (21, 34).

Third, PSAC has unique pharmacological properties. In addition to inhibition by phloridzin (25), furosemide, NPPB [nitro-2-(3-phenyl-propylamino)benzoic acid] (23), and glybenclamide (24)—nonspecific antagonists of channels and carriers—PSAC is unexpectedly blocked by dantrolene, a specific antagonist of sarcoplasmic reticulum Ca²⁺ channels without measurable activity against five other classes of anion channels (G. Lisk and S. A. Desai, submitted for publication). A small secondary screen of dantrolene derivatives subsequently achieved further specificity for PSAC by identifying a nitrophenyl-furan derivative that has higher affinity for PSAC inhibition and no measurable activity against the sarcoplasmic reticulum Ca²⁺ channels (22). Consistent with an essential role for PSAC, this higher-affinity derivative inhibits in vitro parasite growth at lower concentrations than dantrolene.

An important unanswered question is whether other plasmodial species induce similar channels on their host membranes. Previous transport studies using parasites other than P. falciparum have produced mixed answers to this question. One study measured [³H]choline uptake by Plasmodium knowlesi-infected rhesus monkey RBCs and concluded that rather than inducing novel ion channels, this parasite upregulates an endogenous carrier to fulfill the increased demands for this precursor of phospholipid biosynthesis (3). In contrast, another study found that Plasmodium vinckei induces a broad specificity, furosemide-sensitive pathway similar to PSAC and that it also upregulates the endogenous mouse RBC choline carrier (33). Electrophysiological studies of Plasmodium gallinaceum-infected chicken RBCs (36) and of Plasmodium berghei-infected mouse RBCs (20) appear to support upregulation of endogenous channels, although achieved signal-to-noise ratios—calculated from solution compositions and reported seal resistances—were not adequate to rule out PSAC-like channels.

Here, we examined the transport properties of rhesus mon-
key RBCs before and after in vitro infection with *P. knowlesi*, a parasite species chosen in part for its phylogenetic distance from *P. falciparum*. Our osmotic lysis and radioisotope uptake studies revealed increases in organic-solute permeability paralleling those documented with *P. falciparum*. Then, whole-cell and cell-attached patch-clamp methods identified a small-conductance anion channel on *P. knowlesi*-infected rhesus RBCs but absent from uninfected cells. This anion channel showed marked similarities to the *P. falciparum* PSAC, with nearly indistinguishable single-channel conductance, gating behavior, selectivity, and pharmacological properties. We propose that PSAC is highly conserved on these and other plasmodia and that this family of unusual channels is an essential adaptation for survival in the intraerythrocytic environment.

MATERIALS AND METHODS

Parasite culture and osmotic lysis assays. *P. knowlesi* was cultured under in vitro conditions with RBCs collected from healthy rhesus monkeys (*Macaca mulatta*) and RPMI 1640 supplemented with 50 mg/liter hypoxanthine and 20% human serum. In contrast to *P. falciparum*‘s 44 to 48 h cycle in human RBCs, the erythrocytic cycle averaged 24 h. For both parasites, the kinetics of osmotic lysis averaged 24 h. For both parasites, the kinetics of osmotic lysis averaged 24 h. For both parasites, the kinetics of osmotic lysis averaged 24 h. For both parasites, the kinetics of osmotic lysis averaged 24 h. For both parasites, the kinetics of osmotic lysis averaged 24 h. For both parasites, the kinetics of osmotic lysis averaged 24 h.

Electrophysiology. Single-channel and whole-cell patch-clamp recordings of rhesus monkey RBCs were obtained with quartz glass pipettes pulled to tip diameters of <0.5 μm and resistances of 1 to 4 MΩ. Most experiments utilized symmetrical bath and pipette solutions of (in mM) 1,000 choline-Cl, 115 NaCl, 10 MgCl₂, 5 CaCl₂, and 20 Na-HEPES, pH 7.4. Some experiments replaced choline-Cl with equimolar NaCl and identified channel activity with indistinguishable properties. Recordings were filtered at 5 KHz with an eight-pole Bessel filter and digitized at 100 KHz. Seal resistances were typically >100 GΩ, as required for detection of these small-conductance channels (2).

Whole-cell patch-clamp to determine anion selectivity was performed similarly but with a bath solution of (in mM) 500 NaCl, 10 MgCl₂, 5 CaCl₂, 20 Na-HEPES, pH 7.4, and pipette solutions of either 500 NaClO₄ or 500 Na-lactate with 10 MgCl₂, 5 CaCl₂, and 20 Na-HEPES, pH 7.4. Under these conditions, the permeability coefficient of ClO₄⁻ or the lactate anion relative to Cl⁻ can be determined by measuring the reversal potential (E<sub>rev</sub>), the membrane voltage that produces zero current.

Single-channel amplitudes, open probabilities, and dwell distributions were measured with home-written code as described previously (2, 6).

Radioisotope uptake. *P. knowlesi*-infected or uninfected rhesus monkey RBCs were washed and used to measure uptake of [³H]alanine (2 μCi/ml) at a 10% hematocrit in PBS supplemented with 1 mM unlabeled alanine at 22°C. At 5 min intervals, uptake was terminated by transfer of 330-μl aliquots to 1.5 ml of tracer-free PBS with 200 μM furosemide at 4°C and centrifugation at 14,000 × g through dibutyl phthalate. Each time point was measured in duplicate. Cell pellets were digested and counted as previously described (11). Uptake was corrected for extracellular trapping by subtracting the uptake measured at zero time in the presence of furosemide.

RESULTS

Broad increases in permeability after *P. knowlesi* infection. PSAC-mediated permeability changes after infection of human

FIG. 1. Increased organic solute permeability after *P. knowlesi* infection. (A through D) Osmotic lysis of trophozoite-infected rhesus RBCs in buffered sorbitol, isoleucine, alanine, and NaCl solutions (black curves, upper panels). Each lower panel represents an identical experiment with uninfected rhesus monkey RBCs; their lack of measurable lysis reflects their low intrinsic permeability to these solutes. In all panels, red traces reflect the lysis time course in the presence of 200 μM furosemide. Transmittance of 700 nm light though cell suspensions was converted to percent lysis with measurements on identical but fully lysed suspensions of sorbitol-treated infected RBCs or detergent-treated uninfected RBCs. Notice that each of the *P. knowlesi*-induced permeability changes is furosemide-sensitive. (E) [³H]alanine uptake in uninfected RBCs (triangles) or infected RBCs (circles) with or without 200 μM furosemide (red or black symbols, respectively). Uptake was not corrected for parasitemia (6% in this experiment); such corrections would increase the difference between infected and uninfected cells by more than 15-fold. [³H]isoleucine exhibited similar increases in uptake (not shown).
RBCs by *P. falciparum* can be readily identified by an increased osmotic fragility in isotonic solutions of permeant solutes (17). In these solutions, there is net uptake of the permeant solute via PSAC, cell swelling, and selective osmotic lysis of infected RBCs. Lysis of *P. falciparum*-infected human RBCs can be quantitatively and continuously tracked with a simple light-scattering assay that measures the turbidity of the cell suspension (38).

Here, we found that this assay can also be used to follow osmotic lysis of rhesus monkey RBCs with or without *P. knowlesi* infection. These kinetic measurements revealed marked increases in sorbitol, alanine, and isoleucine permeabilities after infection, which could be largely inhibited by 200 μM furosemide (Fig. 1A through C, upper panels). Both uninfected and infected RBCs were stable in isotonic NaCl solutions (Fig. 1D), consistent with sustained low Na\(^+\) permeability despite increases in organic solute permeability. We also confirmed these permeability changes with more conventional \(^{3}H\)-alanine radioisotope accumulation experiments (Fig. 1E). Both

this list of solutes and estimates of their absolute permeabilities parallel previous measurements in the *P. falciparum*-human RBC system (5, 17, 38), suggesting PSAC-like channels on *P. knowlesi*-infected rhesus RBCs.

**Identification and characterization of the *P. knowlesi* PSAC.**

We used electrophysiological methods to probe the mechanistic basis of these increases. Although technically complicated by the somewhat smaller size of rhesus RBCs when compared to human RBCs (35), we successfully achieved high-resistance whole-cell patch-clamp recordings on these cells. In symmetrical bath and pipette solutions containing Cl\(^-\) and *P. knowlesi*-infected RBCs exhibited large whole-cell currents that were absent from uninfected RBCs (Fig. 2). Infected RBC whole-cell measurements revealed a steep voltage dependence with significantly larger currents at negative voltages than at positive voltages despite equal but opposite driving forces. Furosemide abolished these currents, consistent with a broad-specificity channel that mediates the transport of both inorganic anions and the organic solutes evaluated for Fig. 1. In identical whole-cell experiments with uninfected rhesus monkey RBCs, the measured currents were significantly smaller and voltage independent, indicating low permeabilities similar to those of human RBCs (8). (With uninfected RBCs of both species, it is not possible to precisely calculate conductive Cl\(^-\) permeabilities from whole-cell measurements because of errors in measuring their small currents.)

We wondered how these *P. knowlesi*-induced currents compare quantitatively with those induced by *P. falciparum* trophozoites on human RBCs. Because both exhibit larger and more ohmic whole-cell currents at negative imposed membrane potentials (V\(_m\),), we tallied chord conductances between −100 and 0 mV for a number of cells in each system (20 and 21 RBCs for *P. knowlesi* and *P. falciparum*, respectively) (Fig. 2C). These chord conductances were similar despite the phylogenetic distance between the two parasites. We did not perform statistical analyses of these chord conductances because of possible systematic differences in parasite maturity and in pipette access resistances.

We also compared the ion selectivity properties of these parasite-induced currents with bi-ionic experiments that created anion gradients across the RBC membrane while maintaining symmetrical cation concentrations. Under these conditions, anion channels exhibit altered current-voltage profiles with reversal potentials (E\(_{rev}\)) quantitatively linked to relative anion permeabilities (18). With Cl\(^-\) as the predominant extracellular anion, we found that intracellular ClO\(_4^-\) or lactate produced marked and opposing changes in E\(_{rev}\) (Fig. 2D), indicating significantly greater permeabilities for these anions than for Na\(^+\). Our whole-cell studies with both parasites indicate similar anion selectivities (ClO\(_4^-\) > Cl\(^-\) > lactate), consistent with one or more weak binding sites for anions as they cross the host RBC membrane (8).

We next investigated the various similarities between *P. knowlesi* and *P. falciparum*-induced permeability changes with single-channel patch-clamp. In *P. falciparum*, these changes are adequately accounted for by PSAC, whose small conductance requires hypertonic salt solutions for unambiguous detection (2). Because *P. knowlesi* may also induce small-conductance anion channels, we performed cell-attached patch-clamp of infected rhesus monkey RBCs in 1,145 mM Cl\(^-\) solutions.

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**FIG. 2.** Whole-cell patch-clamp measurements. (A) Ensemble of current responses to V\(_m\) values of −100 to +100 mV in 10 mV increments for uninfected rhesus monkey RBC in the whole-cell configuration with 1.0 M choline-Cl in both bath and pipette. (B) Current responses of *P. knowlesi*-infected RBC to the same protocol before or after addition of saturating concentrations of furosemide (left and right groups of traces, respectively). Dashes on the left and right of traces represent the baseline current with zero applied voltage. Notice the currents at negative V\(_m\) are greater than those at corresponding positive V\(_m\) and that furosemide largely abolishes these currents. Scale bars represent 25 ms (horizontal) and 3 nA (vertical) in both panels A and B. (C) Comparison of whole-cell chord conductances between *P. knowlesi*-infected rhesus RBCs (black bar, mean ± standard error of the mean [SEM]) and *P. falciparum*-infected human RBCs (white bar). (D) Anion selectivity determined with bi-ionic experiments with 500 mM Na\(^+\)Cl\(^-\) in the bath and either 500 mM Na\(^+\)ClO\(_4^-\) or 500 mM Na\(^+\)-lactate in the pipette. Black and white bars represent mean reversal potentials ± SEM for experiments with *P. knowlesi*-infected rhesus and *P. falciparum*-infected human RBCs, respectively. The similar reversal potentials indicate similar anion selectivity ratios for these parasite-induced currents and yield mean estimates of permeability ratios relative to Cl\(^-\) of 3.8 and 0.3 for ClO\(_4^-\) and the lactate anion, respectively.
Under these conditions, one or more functional copies of a 20-pS channel were observed in approximately 50% of patches on infected cells (Fig. 3A); this channel was never seen on uninfected rhesus monkey RBCs (23 cells). We measured this new channel’s single-channel amplitudes, open probability, and net ion flux at a range of voltages (Fig. 3B through D) and found that its voltage dependence parallels that of the macroscopic whole-cell currents (Fig. 2B). We also measured the durations of open and closed events at a $V_m$ of $-100$ mV in prolonged single-channel recordings (Fig. 4). These dwell time analyses indicated that the gating behavior of the $P.\text{knowlesi}$-induced channel is indistinguishable from that of the $P.\text{falciparum}$ PSAC. Because gating, single-channel conductance, and voltage dependence constitute a functional signature, we concluded that the ion channels induced by these two parasites are members of a highly conserved family. We suggest that the members of the PSAC family be distinguished by using the parasite species name as a prefix.

Comparative pharmacology of $P.\text{falciparum}$ and $P.\text{knowlesi}$ channels. Our osmotic lysis experiments suggest that the $P.\text{knowlesi}$ PSAC is inhibited by furosemide (Fig. 1). The effects of this inhibitor on the $P.\text{falciparum}$ PSAC have been extensively studied, revealing a single nonluminal binding site with a $K_m$ of 2.7 $\mu$M on the channel extracellular face (2, 7). Strangely, the $P.\text{falciparum}$ PSAC is also inhibited by dantrolene, a specific Ca$^{2+}$ channel antagonist that does not inhibit human anion channels (Lisk and Desai, submitted). We wondered whether the $P.\text{knowlesi}$ PSAC shares these pharmacological properties and used single-channel recordings to explore possible mechanisms. With low concentrations of each of these agents, we found clear inhibitory effects on the $P.\text{knowlesi}$ PSAC (Fig. 5A) that resemble the inhibition with intermediate kinetics described for the $P.\text{falciparum}$ PSAC, indicating another level of conservation for this family of channels.

Are there any differences between these two PSAC channels?
that may have resulted from the phylogenetic distance between
*P. falciparum* and *P. knowlesi*. We used the simple osmotic
lysis assay to survey channel properties and found modest but
reproducible differences in their levels of inhibition by fu-
rosamide. Paired experiments with 4 μM and 10 μM furosimide
consistently produced less robust inhibition of sorbitol-induced
osmotic lysis of *P. falciparum*-infected rhesus RBCs (Fig. 5B and
C). This difference was statistically significant at both concen-
trations (seven trials for each parasite at 4°C). This difference was statistically significant at both concen-
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trations (seven trials for each parasite at 4°C).

FIG. 5. Single-channel pharmacology and a lower furosarnide affinity for the *P. knowlesi* PSAC. (A) Traces reflect current flow through a single
*P. knowlesi* PSAC at V_m of −100 mV without inhibitor or with 4 μM furosarnide, 10 μM furosarnide, or 10 μM dantrolene (top to bottom traces,
respectively). Red dashes on each side of traces reflect the closed-channel level. Scale bars represent 100 ms (horizontal) and 2.0 pA (vertical).
(B and C) Osmotic lysis time courses for *P. falciparum*-infected human and *P. knowlesi*-infected rhesus RBCs in the buffered sorbitol lysis solution
with 0, 4, and 10 μM furosarnide (left to right traces in each panel). Direct comparison of these two groups of unprocessed transmittance
measurements (%T) is possible because both panels used identical hematocris and a common blank. Notice that lysis kinetics are inhibited to a
greater extent for the *P. falciparum* channel at both nonsaturating furosarnide concentrations. (D) Furosarnide dose-response for inhibition of the
*P. knowlesi* PSAC. Sorbitol permeability coefficients (P_s) were calculated from up to eight lysis time courses at each concentration as described
previously (38) and normalized to a value of 1.0 without inhibitor. The solid line represents the best fit to y = K_m/(K_m + x). (E) Closed dwell
distributions from single-molecule recordings of the *P. falciparum* and *P. knowlesi* channels (black and red traces, respectively) in the presence of
10 μM furosarnide. Each histogram tallies approximately 80,000 closing events. For each profile, the peak at a time of >10 ms corresponds to the
population mean for furosarnide-induced closings; this peak is a direct measure of furosarnide unbinding rates from the inhibited channel.
Furosarnide unbinds more quickly from the *P. knowlesi* channel, accounting for its lower affinity there.

DISCUSSION

Plasmodial survival and growth in the cytosolic milieu of
RBCs requires adaptations that allow the trafficking of nutri-
ent precursors from host plasma into parasite compartments.
Work from multiple groups suggests that several nutrients have insufficient permeability in uninfected RBCs to sustain
the known high rates of utilization by the intracellular parasite.
Because PSAC is broadly permeable to these precursors and is
the predominant conductive pathway in the *P. falciparum-*in-
fected human RBC membrane (2), it may function as an es-
tial first step in parasite nutrient acquisition. In our model

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CONSERVED FAMILY OF MALARIA PARASITE-INDUCED CHANNELS

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Studies from multiple groups have produced conflicting observations on whether PSAC represents a parasite-encoded protein trafficked out to the host RBC membrane (2) or a host protein activated by specific or nonspecific parasite activities (12, 21, 37). How do our findings with P. knowlesi-infected rhesus erythrocytes contribute to this debate? Although they do not directly address the genetic basis of PSAC functional activity, our findings require a high level of structural and functional conservation in this family of channels because estimates of the time to the most recent common ancestor are \( \geq 25 \) million years for humans and rhesus monkeys (28) and for P. falciparum and P. knowlesi (14). This level of conservation in spite of the large phylogenetic distances provides compelling yet circumstantial evidence against a protein with an inconsequential physiological role.

Consistent with this proposal, a number of studies from multiple groups used isotope uptake and osmotic lysis measurements to demonstrate increased permeabilities of primate (26, 27), avian (32), and rodent (16, 19, 33) erythrocytes after infection with various plasmodia. Known PSAC inhibitors, whenever tested in these other host-parasite pairs, invariably abrogated these increases, further implicating high-level conservation within this channel family (Fig. 6).

A detailed study of \(^{3}H\)choline uptake by P. knowlesi-infected RBCs (3) failed to detect PSAC-like uptake, seemingly in direct conflict with our findings using the same parasite and host species. In that study, parasite-induced increases in \(^{3}H\)choline uptake were instead attributed to upregulation of endogenous carriers. Experimental differences may well account for the discrepancies with our findings. Their uptake measurements were restricted to choline concentrations of \( \geq 100 \) M, where high-affinity carrier-mediated uptake predominates over uptake via lower-affinity channels such as PSAC. Moreover, they used subtraction of uptake in 1.2 mM \(^{3}H\)choline to correct for extracellular trapping. Because flux through ion channels generally saturates only at much higher solute concentrations, this subtraction systematically excluded detection of channel-mediated uptake in their study. An additional experimental difference, use of in vivo infected monkeys in contrast to our use of parasites cultured in erythrocytes from uninfected monkeys, may also influence the observed upregulation of the endogenous choline carrier.

It is debated whether anion channels other than PSAC exist in P. falciparum-infected human RBCs (12, 21, 32). Some of these proposed other channels, collectively known as “new permeation pathways,” exhibit markedly differing voltage dependences and other biophysical properties. We found no evidence for these “new permeation pathways” on P. knowlesi-infected cells, suggesting that this parasite induces only PSAC-like anion conductances.

PSAC has been recognized as a potentially important and novel target for future antimalarial drug development. Our demonstration of a functional PSAC ortholog on P. knowlesi-infected rhesus RBCs has two important ramifications for drug development programs that target PSAC. The similar pharmacology and mechanisms of channel inhibition indicate that animal malaria models will be suitable for in vivo evaluation of PSAC antagonists identified using laboratory isolates of P. falciparum. These findings also suggest that the PSAC protein is a highly constrained integral membrane protein because
even the biophysical properties of permeation are highly conserved between divergent parasites. We suspect mutations in the channel are constrained by the need to maintain PSAC’s unparalleled selectivity properties and rate of nutrient uptake. In light of these constraints, resistance to antimalarial target- ing PSAC may be harder to acquire than resistance to drugs against less constrained targets inside the parasite compartment.

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REFERENCES