Effects of Cinnamic Acid Derivatives on In Vitro Growth of *Plasmodium falciparum* and on the Permeability of the Membrane of Malaria-Infected Erythrocytes

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Cinnamic acid derivatives (CADs) are known inhibitors of monocarboxylate transport across plasma and mitochondrial membranes. All derivatives were found to inhibit the growth of intraerythrocytic *Plasmodium falciparum* in culture, which is in correlation with their hydrophobic character. Parasites at the ring and trophozoite stages were equally susceptible to the different derivatives. This result could be attributed to their inhibition of the transport of lactate, the major product of parasite energy metabolism. However, unexpectedly, it was found that all derivatives also inhibit the translocation of carbohydrates and amino acids across the new permeability pathways induced in the host cell membrane by the parasite. This impediment correlated strictly with CADs' effect on parasite growth. Parasites residing in cells permeabilized by means of Sendai virus were less susceptible to the different drugs, a result which implies that in addition to the direct effect on parasite viability, the drugs may have inhibited some process in the host cell whose function may be vital for parasite growth. The effect of CADs on the ATP levels in infected cells, in virus-treated cells, and in the two cellular compartments of the infected cell revealed that the drugs caused a significant decline in ATP level in the parasite compartment, while they provoked only a small effect on ATP level in the intact cells and the host cell compartment. These observations suggest that CADs inhibit ATP production in the parasite and its utilization by the host cell.

Malarial parasites develop and propagate asexually inside the erythrocytes of their vertebrate host. They are considered to be homolactate fermenters (25, 26), although recent evidence suggests that they could use their acristate mitochondrion for further oxidation of glycolytic products (1, 9). The glucose consumption and lactic acid production of malaria-infected erythrocytes are increased as the parasite develops up to 100-fold compared with those of uninfected cells (22, 24). Nearly all of the surplus lactic acid production must originate from parasite metabolism, since acceleration of host cell glycolysis is restricted by the maximal activity of the rate-limiting enzyme hexokinase (15). Doubtlessly the parasite must have very efficient means for elimination of lactic acid, and this must be done by excretion, since, as far as we know, the parasite is incapable of gluconeogenesis. Virtually nothing is known about the way the parasite disposes of lactic acid. By deduction from observations of other cells and organelles, lactate can be transported by free diffusion of lactic acid, by a specific monocarboxylate carrier, or via a lactate-H⁺ symport (6, 13). Whereas in human erythrocytes lactate is transported by three parallel mechanisms, i.e., lactate-H⁺ symport, the anion carrier, and diffusion of lactic acid (presented in the order of their relative contributions at physiological lactate concentrations), the overall transport capacity is relatively small and cannot tally with the high rates of lactic acid production by the parasite (4). Hence, this capacity must be augmented by modulation of native transport systems or the new permeability pathways that the parasite induces in the host cell membrane (10) must be able to accommodate this compound as well.

Derivatives of cinnamic acid (CADs) are well-characterized inhibitors of lactate transport either by means of the lactate-H⁺ symport across the erythrocyte membrane or by the monocarboxylate carrier across the mitochondrial membrane (12, 13) and can therefore be used to investigate the transport aspect of lactate metabolism in malaria-infected erythrocytes. It is expected that they would inhibit the excretion of lactic acid from the parasitized erythrocyte, and, to the extent that the parasite's mitochondrion is instrumental in energy production, they could inhibit ATP generation. In both cases, they are anticipated to inhibit parasite growth and to be helpful in elucidating the bioenergetic role of the parasite's mitochondrion.

MATERIALS AND METHODS

Parasite cultures. The FCR3 strain of *Plasmodium falciparum* was cultivated in either O⁺ or A⁺ washed human erythrocytes at 37°C in RPMI 1640 medium (GIBCO) supplemented with 10% (vol/vol) AB⁺ or A⁺ heat-inactivated human plasma—25 mM NaHCO₃ (N-[N-(2-hydroxyethyl)piperazin-1-yl]2-ethanesulfonic acid)–32 mM NaHCO₃–10 mM glucose. The growth medium in 150-cm² culture flasks was replaced daily and then gassed with a mixture of 90% N₂, 5% CO₂, and 5% O₂.

Erythrocytes harboring parasites at mature stages (trophozoites and schizonts) were concentrated from culture by using gelatin floation (16) or Percoll-alanine gradient centrifugation essentially as described in reference 19, except that sorbitol was replaced with the less toxic amino acid alanine (3% wt/vol). After separation, cells were washed twice in wash medium (i.e., growth medium without plasma) and allowed to recover for 2 h under culture conditions. Cells were counted in a cell counter (Analys Instruments, Stockholm, Sweden), and the percentage of infected cells

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(parasitemia) was determined by microscopic inspection of Giemsa-stained thin blood smears.

**Preparation of "free parasites."** The interaction of infected cells with Sendai virus causes specific lysis of the host cell due to the insertion in its membrane of pores that can admit solutes having molecular weights of 1,600 or less. The parasites that are now exposed to the extracellular medium remain viable for several hours (17, 18). Infected cells were incubated at 2.5% hematocrit in wash medium with Sendai virus virions (25% 50 μg of protein per ml, equivalent to 400 to 600 hemagglutination units) for 6 min at 37°C and centrifuged for 30 s in an Eppendorf microcentrifuge. The resulting supernatant and pellet that represent, respectively, the host cytosol and the parasite compartment were used for the analysis of ATP content (see below), and the "free parasites" (still engulfed by the permeabilized host cell membrane) were used to test the effect of CADs on their viability.

**Effect on parasite growth.** Cultures of parasites were synchronized to the ring stage by using isosmotic saline (20). Cells were suspended in RPMI 1640 medium containing 10 mM NaHCO3 to 1% parasitemia and 2% hematocrit and distributed into wells of a 24-well microtiter plate (1 ml per well). Inhibitors and [3H]hypoxanthine (10 μCi/ml) were added to half the number of wells to the indicated concentrations (see Fig. 2). After 24 h of incubation at 37°C in an atmosphere of the gas mixture, aliquots of 200 μl were transferred in triplicate to a 96-well plate and harvested with a cell harvester (Dynatech, Inc.). The inhibitors and [3H]hypoxanthine were added to the remaining wells. After an additional 8 h of culture, the cells were harvested. The filters were washed with distilled water, dried for 2 h at 60°C, and transferred into toluene-based scintillation fluid for counting of radioactivity. 50% inhibitory doses (IC50s) were calculated as previously described (5).

In another experiment, intact and virus-treated trophozoite-infected cells (3% parasitemia, 1% hematocrit) were suspended in the same growth medium and distributed into wells of a 24-well microtiter plate. The cells were harvested after 8 h of incubation with [3H]hypoxanthine and inhibitors and processed as described above.

**Transport measurements by using substrate-mediated hemolysis.** The half-time of substrate-mediated hemolysis of infected cells is inversely proportional to the permeability of the host cell membrane to that substrate. The time course of hemolysis was assayed as previously described (28). Flux was initiated by adding 20 μl of the cell suspension (10 to 20% parasitemia, 25% hematocrit in phosphate-buffered saline [145 mM NaCl and 10 mM sodium phosphate, pH 7.4, PBS]) to wells of a U-bottom 96-well plate (Nunc) containing 100 μl of the flux medium (200 mM substrate, 40 mM NaCl, 5 mM sodium phosphate [pH 7.4], and 0.5 mM 4,4'-dinitrostilbene-2,2'-disulfonic acid [DNDs], to inhibit transfer of solutes that could be mediated by the anion transport system), with or without inhibitor. Hemolysis was stopped at different time intervals by adding 60 μl of a 0.5 M sucrose solution. At the end of the flux experiment, the plate was centrifuged (2,000 × g for 5 min) and 100-μl samples of the supernatants were withdrawn, transferred to another plate, and read at 405 nm with a Bio-Tek enzyme-linked immunosorbent assay (ELISA) ready to assess the amounts of hemoglobin released because of cell lysis. All of the above steps were carried out at room temperature.

**Influx of radiolabeled substrates.** Normal erythrocytes or cell preparations containing more than 80% trophozoite-infected cells were used. Prior to influx assay, the cells were suspended at 10% hematocrit in wash medium or PBS. The cells were preincubated for 5 to 10 min with 0.5 μM DNDS in the presence or absence of inhibitor. Influx was initiated by the rapid mixing of an equivalent volume of the same medium containing 0.5 mM DNDS, cold and radioactive substrate, and the same inhibitor (loading solution). At different time intervals, triplicates of 50 μl of the cell suspension were overlaid on top of 100 μl of ice-cold dibutyl phthalate in a 0.4-ml polyethylene microcentrifuge tube and centrifuged for 15 s in a Beckman microcentrifuge. Time zero data were obtained as follows. The dibutyl phthalate in the tube was overlaid with 25 μl of ice-cold loading solution, and the tube was placed horizontally in the microcentrifuge. A cell suspension (25 μl) was carefully pipetted onto the tube wall, avoiding mixing with the loading solution. The centrifuge was then activated, driving the cells through the loading solution and then through the phthalate layer. The dwelling time in the presence of radiolabel was about 15 ms (8a). After centrifugation, the tip of the tube containing the cell pellet was cut off and placed in a tube containing 0.5 ml of distilled H2O to lyse the cells. Residual dibutyl phthalate was removed by centrifugation of the lysate for 2 min. An aliquot of 475 μl of the lysate was mixed with 25 μl of 100% (wt/vol) trichloroacetic acid and centrifuged, and 400 μl of the clear harvest liquid was taken for radioactivity counting. The remaining 25 μl of lysate was diluted 1:4 with distilled water in a 96-well plate, and hemoglobin was determined from its A405 by using a Bio-Tek ELISA reader. Results in disintegrations per minute of absorbance were converted to millimoles per liter of cell volume by using specific activity of the substrate and the related number of cells obtained from the hemoglobin absorbance.

**Determination of ATP.** Normal erythrocytes were under culture conditions for 24 h, and trophozoite-infected erythrocytes were separated from culture by using the gelatin method. Virus-treated and intact cells were incubated in the presence or absence of 3 mM a-FC or 0.5 mM UK-5099. After 2 h of incubation, aliquots of 50 μl were withdrawn from virus-treated and normal erythrocyte suspensions and added to 50 μl of 10% trichloroacetic acid. ATP in the clear supernatant was determined after centrifugation for 1 min in an Eppendorf centrifuge. For compartment analysis of ATP, 0.5-ml aliquots from the suspension of intact infected erythrocytes that were incubated for 2 h in the absence or presence of inhibitors were transferred to Eppendorf tubes and treated with Sendai virus as described above in the presence of ATPase inhibitors (2 mM EDTA, 0.5 mM L-Cl) and 50 μM adenosine kinase inhibitor (diadenosine pentaphosphate). ATP was determined in the two compartments (see Results).

ATP was measured by using the bioluminescence assay (17, 18). Diluted samples (200 μl) were added to 40 μl of luciferin-luciferase reagent (Lumac 3M) reconstituted with 25 mM HEPES (pH 7.75), mixed rapidly, and placed in a Lumac 3M Biocounter, and the constant level of light emission was recorded. Finally, 5 μl of 1 × 10−6 M ATP standard was added and the increase in light emission was recorded again.

The amount of ATP in the sample was calculated from the increment of light emission upon addition of sample and ATP standard and taking into account dilutions, the concentration of the ATP standard, and the cell concentration in the original cell suspension.

**Chemicals.** α-Fluorocinnamylamine (α-FC), α-cyano-4-hydroxycinnamylamine (α-C4HC), and α-cyano-3-hydroxycinnamylamine (α-C3HC) were purchased from Sigma Chemical Co. α-Cyano-β-(1-phenylindol-3-yl) acrylate (UK-5099) was obtained from
been used to study parasite growth. Parasites were grown in culture in the presence of the indicated concentrations of inhibitors and 10 μCi of \[^{3}H\]hypoxanthine per ml. After 24 h of incubation at 37°C, cells were harvested and the \[^{3}H\]hypoxanthine incorporated was determined as described in Materials and Methods. Data are given as percent incorporation of hypoxanthine into cells exposed to the corresponding concentration of inhibitor relative to control cells (no inhibitor). Symbols: ⮑, UK-5099; ⮜, α-C3HC; ▲, α-FC; △, α-C4HC. The IC\textsubscript{50} ± the standard deviations (millimolar) for the different inhibitors were as follows: UK-5099, 0.064 ± 0.006; α-C3HC, 0.258 ± 0.025; α-FC, 0.612 ± 0.084; α-C4HC, 1.126 ± 0.005.

**RESULTS**

CADs (Fig. 1) are well-known inhibitors of carrier-mediated lactate and pyruvate transport across cellular and mitochondrial membranes (12, 13). All CADs tested arrested parasite growth (Fig. 2). They were found to be equally inhibitory to all stages of parasite development (Table 1), and the relatively apolar derivative UK-5099 was invariably found to be the most potent inhibitor.

CADs also inhibited \[^{3}H\]hypoxanthine incorporation into free parasites (Table 1), but the IC\textsubscript{50}S obtained with virus-treated cells were higher than those observed with intact cells. This result implies that CADs act directly on the parasite, but in addition, they may have inhibited some process in the host cell whose function may be vital for parasite growth.

**Effect of CADs on solute transport across the host cell membrane.** The substrate-induced hemolysis technique has been used to assay the effect of CADs on transport across the host cell membrane (10). As depicted in Fig. 3, UK-5099 at 0.1 mM markedly inhibited the transport of glucose and glycine and to a lesser extent that of lactate. The dose dependence of this inhibitory effect and the relative potencies of the various CADs were determined by deriving the half-time of lysis induced by isosmotic solutions of sorbitol.

### TABLE 1. Effect of CADs on the ring and trophozoite stages of parasite development and on parasite development in intact and virus-treated cells

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC\textsubscript{50} of CADs for\textsuperscript{a}:</th>
<th>Ring-stage parasites</th>
<th>Trophozoite-stage parasites</th>
<th>Intact cells</th>
<th>Virus-treated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK-5099</td>
<td>0.052 ± 0.010*</td>
<td>0.104 ± 0.009</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>α-C3HC</td>
<td>0.212 ± 0.044</td>
<td>0.275 ± 0.010</td>
<td>0.333 ± 0.019</td>
<td>0.573 ± 0.017</td>
<td></td>
</tr>
<tr>
<td>α-C4HC</td>
<td>0.862 ± 0.075</td>
<td>0.970 ± 0.001</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>α-FC</td>
<td>1.165 ± 0.165</td>
<td>1.093 ± 0.018</td>
<td>0.856 ± 0.174</td>
<td>0.996 ± 0.004</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values are expressed as millimolar concentrations ± standard deviations. Control values of incorporation into virus-treated cells were 50 to 60% those of incorporation into intact cells. ND, not determined.
The calculated initial rates \([S]_o \cdot k\) of influx of lactate, sorbitol, 2-deoxyglucose, and glycine were reduced by 90, 74, 50, and 87%, respectively, by the specific concentration of CADs used, indicating that their transport across the host cell membrane is markedly inhibited at physiological solute concentrations.

**EFFECT of CADs on the level of ATP in infected cells.** Since CADs were found to inhibit parasite growth and transport of solutes that may affect the energy metabolism of the parasite, e.g., glucose, we have tested their effect on the ATP levels in infected intact and virus-treated cells. As shown in Fig. 5, \(\alpha\)-FC, \(\alpha\)-C3HC and \(\alpha\)-C4HC prevented the decline of ATP levels in intact infected cells with time, which is due to the depletion of glucose from the medium. UK-5099, on the other hand, caused a considerable decline of ATP levels in these cells. Parallel experiments done on free parasites, e.g., virus-treated cells, resulted in a decrease of ATP in the
TABLE 2. Effect of CADs on ATP levels in normal and infected erythrocytes

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Virus-treated cells</th>
<th>Host compartment</th>
<th>Parasite compartment</th>
<th>Normal erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47</td>
<td>74</td>
<td>63</td>
<td>122</td>
</tr>
<tr>
<td>α-FC (3 mM)</td>
<td>24</td>
<td>72</td>
<td>45</td>
<td>122</td>
</tr>
<tr>
<td>UK-5099 (0.5 mM)</td>
<td>20</td>
<td>59</td>
<td>20</td>
<td>110</td>
</tr>
</tbody>
</table>

parasite, but only the latter affected somewhat the host cytosol ATP. Neither drug had any effect on the ATP level in uninfected erythrocytes.

**DISCUSSION**

Asexual malaria parasites developing inside the erythrocytes of their vertebrate host are considered to be homolactate fermenters (24), although some metabolic energy may be produced by the parasite's mitochondrion (1, 7, 8, 9). The large amounts of glucose consumed by the parasite (28) result in the production of concomitant quantities of lactic acid (22-24). This must be disposed of in order to prevent acidification of the parasite's cytoplasm and to permit the regeneration of NAD. CADs inhibit the transport of monocarboxylates across erythrocyte and mitochondrial membranes (12, 13). In erythrocytes they are competitive and noncovalent, whereas in mitochondria they are noncompetitive because of thiol modification (13). For this reason, replacement of the α-cyano group by a fluorine atom (as in α-FC) has no consequence for inhibition of transport across erythrocyte membranes but abrogates inhibition of pyruvate transport in mitochondria (12). Therefore, we presumed that CADs could inhibit parasite growth and propagation through blocking of lactate egress and that selective use under well-developed experimental conditions could further elucidate the putative contribution of the parasite's mitochondrion to energy production.

Our results clearly demonstrate that CADs inhibit parasite growth and that they are equally effective at the young (ring) and the mature (trophozoite) stages of parasite development. While the IC₅₀ for α-FC and α-C4HC are substantially higher than their respective Ki values for inhibition of lactate and pyruvate transport across the erythrocyte membrane, those for UK-5099 and α-C3HC are very similar to their respective Ki values (13). Since in the isolated rat liver mitochondrion, inhibitors are much more potent than in intact cells (14), these discrepancies may be due to the demonstrable binding of CADs to albumin (14) which is present in the parasite culture medium. The impairment of parasite growth by CADs could be due to inhibition of lactate transport or of mitochondrial respiration. It must be emphasized that since CADs are also noxious to host cells, they could not be proposed as novel antimalarial drugs. They have been used in the present works as tools for the investigation of lactate disposal and for the detection of possible new targets for chemotherapeutic onslaught.

By using physiological lactate concentrations, a huge increase in the permeability to lactate in infected cells has been observed. This suggests either that the constitutive monocarboxylate carrier is somehow activated or that the increased influx is mediated by the new permeation pathways that are induced by the parasite in the membrane of its host cell (11). It is clear that the total lactate transport presence of all drugs (Fig. 6). The relative potency of the drugs is directly related to their relative hydrophobicity (12). In an attempt to resolve these apparently conflicting results, i.e., lack of effect in intact cells versus inhibition in virus-treated cells, we have treated intact cells with the drugs and thereafter determined the ATP levels separately in the host and the parasite compartments (Table 2). It is clear that both α-FC and UK-5099 caused a decline of ATP levels in the

![Figure 5: Effect of CADs on ATP levels in intact trophozoite-infected erythrocytes. Trophozoite-infected cells were enriched from culture by gelatin flotation and returned to culture conditions for 2 h. The cells were gassed and incubated at 37°C in growth medium supplemented with 10 mM NaHCO₃, 50 μM coenzyme A, and the indicated inhibitors. Aliquots were taken at different time intervals and added to an equal volume of 10% trichloroacetic acid. ATP concentration was determined by using the luciferin-luciferase coupled assay as described in Materials and Methods. Results are given in micromoles of ATP per 10⁹ cells. Symbols: ○, control; Δ, α-FC (3 mM); C, α-C4HC (3 mM); +, α-C3HC (3 mM); △, UK-5099 (1 mM).]

**TIME OF INCUBATION (hrs)**

![Figure 6: Effect of CADs on ATP levels in virus-treated trophozoite-infected erythrocytes. Experimental conditions were as detailed in the legend to Fig. 5, except that the cells were permeabilized with Sendai virus as described in Materials and Methods. Results are given in micromoles of ATP per ml of compartment volume by taking into consideration that the relative volume occupied by the parasite is 0.35. Symbols and inhibitor concentrations are same as for Fig. 5.](http://aac.asm.org/)

![Graph](http://aac.asm.org/)

![Table 2](http://aac.asm.org/)
capacity of human erythrocytes cannot sustain the increased lactate production of infected cells (6, 13). An increment in the permeability to this metabolic waste product seems therefore indispensable for permitting parasite growth inside the erythrocyte. An insight into the mechanistic nature of the increased lactate permeability is hinted at by the effect of CADs. Their lesser restraint on lactate-mediated lysis than on lactate influx at physiological concentrations may be related to the competitive nature of CADs, implying that the constitutive carrier of the host cell membrane has been activated by the presence of the parasite. But an alternative interpretation is also possible. The membrane of normal erythrocytes contains constitutive carrier-mediated transport systems that translocate glucose (29), lactate (6, 13), and glycine (27). That of the malaria-infected cell displays new permeability pathways accessible to these and other solutes (10), including sorbitol (10). By their intrinsic nature, membrane carriers are saturable, while the new pathways are not. Hence, one could expect a relatively large contribution of the constitutive pathways to overall transport at lower solute concentrations, while at higher (e.g., isosmotic) concentrations, the contribution of the native transporter could be much smaller. The smaller inhibitory effect of CADs on lactate-induced lysis, for which lactate concentration is 30-fold larger than in the tracer experiments, can be explained on this basis.

The inhibitory effect of CADs on the translocation of carbohydrates and amino acids across the host cell membrane was rather unexpected. The transport of these solutes is mediated by parasite-induced new permeability pathways that were attributed to structural defects introduced into the host cell membrane by inserted parasite polypeptides (11) or by these proteins themselves (3). Unlike the case with lactate, CADs were equally effective in inhibiting solute-mediated lysis and influx at physiological concentrations. Clearly, inhibition was not competitive. The pattern and selectivity of inhibition by CADs are reminiscent of those observed with bioflavonoids (28), which were shown to act on the cytoplasmic face of the membrane (2), and with pyridoxalhydrazone derivatives that are iron chelators (14a).

Both types of compounds are less inhibitory to lactate transport than to sorbitol or glycine transport across the new permeability pathways. Structure-activity relationships indicate that the effectiveness of CADs is related to their relative hydrophobicity. This can be due either to their ease of penetration into the cell or to the nature of their interaction with the transport agency. We favor the second alternative since we could not observe any time-dependent increment of CAD inhibition that would be expected from differential rates of CAD translocation. The variable chemical character of these different inhibitors seems to support the notion that the new permeability pathways are due to structural defects (11) rather than to the induction of specific transport agencies or the modulation of native transporters (3).

The effects of CADs on the ATP levels of infected cells are rather ambiguous. While they do not influence the ATP level in uninfected erythrocytes (except for UK-5099) and barely affect that in the cytosol of the infected cell, they provoke a drop in the parasite ATP level both in intact and in virus-treated cells. Since the parasite supplies ATP to its host cell (18), a decline in the parasite ATP level should have resulted in a drop of the host cytosol ATP level. The absence of this expected decline may imply inhibition of ATP-consuming processes by CADs in the host and/or their acceleration in the parasite. We could not find any hint in the literature of a possible impediment of either Na⁺-K⁺-ATPase or Ca²⁺-ATPase, whose activities account for about 80% of ATP consumption in normal erythrocytes (21). The decrease in the parasite ATP level could have been related to inhibition of pyruvate transport across the mitochondrion. However, α-FC, which does not interfere with this process in mammalian mitochondria, lowered the ATP level in the parasite cytosol almost as efficiently as UK-5099. Since both compounds inhibit the transport of lactate and 2-deoxyglucose across the parasite membrane in intact infected cells, they could act both by metabolic deprivation and/or blockage of waste disposal and by inhibition of mitochondrial ATP generation. This would require that the parasite’s mitochondrial pyruvate transporter is different from that of the mammalian organelle in its responsiveness to α-FC. Partial inhibition of glucose and/or lactate transport across the host cell and the parasite membranes is probably the major cause for their inhibition of parasite growth. But an effect on ATP generation by the parasite’s mitochondrion cannot be excluded, thus highlighting the role of this organelle in the parasite’s energy metabolism.

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ADDITIONAL IN PROOF

The transport of L-lactate across the membranes of the parasite and the infected erythrocytes has been recently characterized (J. Kanaani and H. Ginsburg, J. Cell. Physiol. 149:469–476, 1991). Transport of radiolabelled L-lactate across the host cell membrane was shown to increase ca. 600-fold compared with uninfected erythrocytes. It showed no saturation with radiolabelled L-lactate and was inhibited by CADs but not by the SH reagent p-chloromercuri phenyl sulfonic acid (PCMBs). These results suggest that L-lactate is translocated through CAD-inhibitable new pathways induced in the host cell membrane by parasite activity, probably by diffusion of the acid form and through a modified native monocarboxylate:H⁺ symporter. Continuous monitoring of extracellular pH changes occurring upon suspension of infected cells in isosmotic Na-lactate solutions indicates that part of the lactate egress is mediated by anionic exchange through the constitutive, but modified, anion exchanger. The transport of L-lactate across the parasite membrane is rapid, nonsaturating, and insensitive to either CADs or PCMBs or to the presence of pyruvate. L-Lactate uptake increased transiently when external pH was lowered and decreased when ΔpH was dissipated by the protonophore carbonylcyanide m-chlorophenyl hydradzone. These results are compatible with L-lactate crossing the parasite membrane either as the undissociated acid or by means of a novel type of lactate/H⁺ symporter.

REFERENCES


16. Ibeancho, E. Personal communication.


