Biochimica et Biophysica Acta 861 (1986) 194–196
Elsevier

BBA 70337

BBA Report

Selectivity properties of pores induced in host erythrocyte membrane by *Plasmodium falciparum*. Effect of parasite maturation

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(Received 21 May 1986)

Key words: Membrane permeability; Pore selectivity; Malaria; Erythrocyte membrane; (*P. falciparum*)

The intraerythrocytic malarial parasite permeabilizes its host cell membrane by inducing pore-like pathways which mediate the passage of nonelectrolytes and anions. In the present work we show that, although the permeability increases with parasite maturation, the selectivity of the pores to various solutes is essentially preserved, suggesting that the number of pores increases without any alteration in their intrinsic solute conductance.

The malarial parasite completes its asexual cell cycle within the erythrocytes of its host. While residing inside the host cell, it affects the permselectivity of the erythrocyte membrane, rendering it highly permeable to small nonelectrolytes [1–5] and to anions [6,7] and somewhat more permeable to cations [8–10]. The new permeability pathways in human erythrocytes infected with *Plasmodium falciparum* display the properties of anion selective pores, having an apparent diameter of 0.7 nm [4]. Recently, we have shown that the parasite-induced permeability appears 6 h after the invasion of the erythrocyte by the parasite and that it increases with the intracellular development of the parasite [11]. Based on the demonstrable dependence of the permeability increases on parasite protein synthesis, we have suggested that the new permeability pathways could be associated with parasite polypeptides which are inserted in the host-cell membrane [11]. Thus, the observed increase in permeability with intraerythrocytic development of parasites could result either from an increase in the number of pores or from an increase in the intrinsic conductance of the pores, or from both. In the second case, one would expect also a concomitant change in the selectivity of the pore towards different permeating solutes.

In the present work we have tested the permeability of the membrane of *P. falciparum*-infected human erythrocytes to different solutes as a function of parasite developmental stage. We show that the relative permeability to different solutes remains unaltered in spite of a dramatic increase in their absolute rates of permeation.

The FCR-3 strain of *P. falciparum* was cultivated in RPMI-1640 medium supplemented with 20 mM glucose, 25 mM Hepes, 32 mM $NaHCO_3$ and 10% (v/v) heat-inactivated AB^+ plasma, using fresh A^+ or O^+ saline-washed red blood cells at 2.5% hematocrit. The growth medium was changed daily followed by gassing with a mixture of 90% N_2/5% CO_2/5% O_2. Cultures were kept synchronous by occasional treatment with isotonic sorbitol [12]. Determinations of parasite stage and of the percentage of infected cells (parasitemial) were

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performed by microscopic inspection of Giemsa-stained thin blood smears. Infected red blood cells harboring young parasite stages (6–11 h post invasion) were isolated by the Percoll-sorbitol gradient method [11], washed of Percoll and returned to culture conditions. After 1, 8 and 20 h, the initial rate of influx of various solutes (at 1 mM) into infected cells was determined at 37°C. Linear rates of uptake were maintained for about 6 min at the ring stage and for up to 1.5 min in cells infected with the more mature trophozoite stage. Uptake into uninfected cells was measured in parallel for 6 min. Results shown in Fig. 1 indicate that the absolute rate of transport of all solutes increased with parasite development. A close inspection of the results, revealed that for those substrates which showed limited permeation into uninfected cells, e.g., alanine, glycine, sorbitol and *myo*-inositol, the rate of uptake increased 2–3.5-fold during the transition from early to late ring and 4.5–6-fold after reaching the trophozoite stage. On the other hand, glutamine, isoleucine and fructose displayed measurable uptake also in uninfected cells. For them, the major increase in uptake (3.1–6.8-fold) was observed during the transition to late ring, while a relatively smaller increase (2.5–3.6-fold) occurred upon maturation of late rings into trophozoites. This apparent discrepancy emerges from the contribution of the native transport systems of the host-cell membrane to the overall measured transport. However, after this contribution is subtracted, it becomes evident that the basic selectivity pattern of the parasite-induced permeability pathways [4] was largely preserved throughout parasite maturation. Taken in toto, the above observations agree with the notion that it is the number of pores which increases with parasite development and not their intrinsic capacity of transport. Support for this concept has recently been obtained by direct measurements of the anion conductances in whole cell patches of *P. falciparum*-infected cells (Stutzin, A. and Cabantchik, Z.I., unpublished data).

A complementary approach to the characterization of the pores according to their solute selectivity, is the test of the susceptibility of the pores to various inhibitors. This is based on the idea that if the nature of the pores does not change with parasite maturation, the pores should show similar

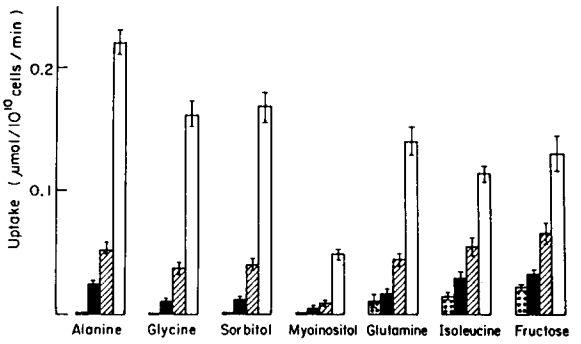

Fig. 1. Initial rate of uptake of different solutes into *P. falciparum*-infected red blood cells as a function of parasite development. Cells infected with young ring stage parasites were isolated from synchronous cultures by the Percoll-sorbitol method [11] and returned to culture conditions. After 1 h (early rings), 8 h (late rings) and 20 h (trophozoites), cells were washed once in phosphate-buffered saline (5 mM $NaHPO_4$/150 mM, NaCl (pH 7.4)) and resuspended in the same buffer to 10% hematocrit. Uptake of radiolabeled substrates was initiated by mixing equal volumes of 2 mM substrate (8 μCi/ml) and cell suspension, prewarmed to 37°C. Aliquots in triplicate were taken at different time intervals and spun through *n*-dibutyl phthalate. The cell pellet was lysed in distilled water, radioactivity and hemoglobin concentration were determined in the lysate and initial rates of uptake were calculated as previously described [15] taking into account the extent of hemoglobin degradation by the parasite at the different developmental stages. Initial rates of uptake into uninfected cells were similarly determined. Sample designation from left to right for each substrate: uninfected, early rings, late rings, trophozoites.

susceptibility to inhibitors. We have used two nonspecific transport inhibitors, phlorizin and its aglycon, phloretin, as they were previously shown to inhibit this pathway in erythrocytes harboring mature parasites [7,13]. The results shown in Fig. 2, indicate that phloretin inhibits the uptake of sorbitol equally well at the ring and the trophozoite stage. On the other hand, phlorizin which was effective only on mature forms, was shown to affect transport by binding to the cytoplasmic side of the pores (Kutner et al., unpublished data). Thus, the apparent lack of susceptibility of rings to this drug resulted from the limited permeability of rings to phlorizin, which prevented the drug from gaining access to the host cell cytoplasm.

Using the initial rates of uptake presented above, we calculate the number of pores as fol-

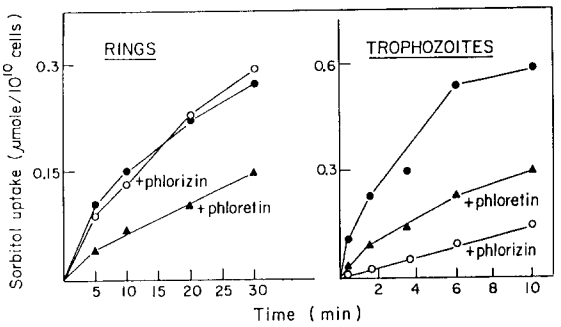

Fig. 2. Stage-dependent effect of phlorizin and phloretin on sorbitol uptake by infected cells. Red blood cells infected with early ring and trophozoite stage parasites were obtained by the Percol-sorbitol method and post-cultivation as described above. Sorbitol uptake in the presence and absence of 100 μM phlorizin or phloretin, was assayed as described in the legend to Fig. 1.

lows: First the permeability coefficient, P (in cm/s), is calculated using the relationship

$$\text{flux} = P \cdot A \cdot dC$$

where A is the area of 10^{10} cells (the area of a single cell being 140 μm^2) and dC is the concentration of the solute, i.e., 1 mM, assuming that the solute is initially absent from the intracellular compartment. Then, the diffusion coefficient D' (in cm^2/s) is computed using the relationship

$$D' = P \cdot dX$$

where dX is the length of the pore, taken as $4 \cdot 10^{-7}$ cm, equivalent to the thickness of the membrane. We then compute the fractional area of the membrane which is open to free diffusion from the ratio D/D' where D is the diffusion coefficient for the solute in water, assuming that the pores are filled with water. Thus, the membrane area open for diffusion in $Å^2$/cell is calculated and divided by the area of a single pore (assuming a radius of 5 Å from the solute exclusion data of Ginsburg et al. [4]) to give the number of pores per cell. Finally, this latter number must be corrected for the 'excluded area' effect by dividing it by $(1 - r/R)^2$, where r is the radius of the permeating solute and R is the that of the pore [14]. From these calculations one obtains 8–16 pores/cell at the trophozoite stage and about a tenth of that number at the early ring stage.

This work was supported in part by NIH grant AI 20342.

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