14C-Desferrioxamine B: Uptake into erythrocytes infected with Plasmodium falciparum

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Abstract. Plasmodium falciparum-infected human erythrocytes (early trophozoite stages) and non-infected erythrocytes were incubated in 1.7 mM 14C-desferrioxamine B (specific activity 1 μCi/2.6 mg desferrioxamine B). After 270 min the cells were washed and the radioactivity was measured in the cell pellet and, after lysis, in cytoplasm and membranes. The results indicate that Desferrioxamine B can the red blood cell and pass through the parasite membrane and that the parasites are killed by the intracellular action of the chelator.

Introduction
All organisms probably require iron for growth (Neilands 1981). This includes the intraerythrocytic forms of the malarial parasites which are inhibited by the microbial iron chelator Desferrioxamine B (DFO) in vitro (Raventos-Suarez et al. 1982) and in vivo (Fritsch et al. 1985). However, it is not known whether this effect is due to the chelation of intracellular or extracellular iron. Robbins and Pederson (1970) described an uptake of the hydrophilic DFO molecule into HeLa cells, and Laub et al. (1984, 1985) found an accumulation of the chelator in rat hepatocytes and macrophages. Using 14C-DFO, we tested whether DFO is taken up by P. falciparum-parasitized and non-parasitized human red blood cells.

Materials and methods
Parasites and culture
Plasmodium falciparum (FCB strain, knobby) were grown in six well plates (Falcon) using the candle jar method (Jensen and Trager 1977). Group A erythrocytes were cultured in RPMI 1640 medium (Gibco) containing 10% human A/AB serum, 100 μg/ml gentamycin

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Treatment of cultures with DFO

Desferrioxamine B (as methyl sulfonate, DFO) was dissolved to 20 mM in serum-free medium (stock solution). To prepare the Fe$^{3+}$/DFO complex, equimolar quantities of DFO and FeCl$_3$ were dissolved in H$_2$O, mixed, and buffered to pH 7.4 with serum-free medium.

Synchronized ring forms and early and mature trophozoites were cultured in a growth medium containing 50 µM DFO. After 10 h the cells were washed three times in complete medium (1,000 g, 5 min, room temperature) and cultivation was continued in DFO-free medium. Parasite growth was assessed by Giemsa stained blood smears.

The synchronisation resulted in a parasitemia of 7.2% of developing ring forms. At the early trophozoite stage, 14C-DFO (as methyl sulfonate) was added to the final concentration of 1.7 mM and the cells were incubated for 270 min. The large amount of DFO was necessary due to its low specific activity (1 µCi/2.6 mg DFO). Uninfected red blood cells with 14C-DFO and infected cells served as controls; each test was duplicated. Parasite growth was assessed every hour over a period of 12 h by Giemsa stained blood smears. After incubation, labelled infected and labelled uninfected red blood cells were washed by centrifugation. The pellets were diluted in 10 ml of serum-free culture medium containing 1 mg/ml unlabelled DFO (to avoid high concentration gradients between intra- and extracellular spaces) and centrifuged at 1,000 g and 4°C for 5 min. The washing procedure was repeated twice and 500 µl samples of each supernatant were reserved for scintillation counting. The cell pellets were divided into two parts, the first of which was prepared for scintillation counting, the second lysed in a 14-fold volume of a hypotonic sodium phosphate buffer (5 mM, pH 7.4), followed by 5 min sonication at 0°C (Bandelin Sonorex sonication bath). The resulting suspension was centrifuged at 100,000 g and 4°C for 30 min.

To determine the radioactivity, the blood and supernatant samples (100 or 500 µl each) were mixed with 500 µl 1 N NaOH, followed by 500 µl of concentrated H$_2$O$_2$ and 15 ml Aquiluma Plus (Lumac, Basel, Switzerland). To eliminate excess H$_2$O$_2$ and avoid chemoluminescence, the samples were degassed in a sonication bath at 60°C for 15 min. Samples were measured in a Liquid Scintillation Counter (Beckman LS 6800) for 10 min or to a degree of accuracy of 2 sigma, using a calibration curve and the external standard method. Further control was achieved by the internal standard method (addition of 100,000 dpm of 14C per vial).

Results

The growth of *P. falciparum* in vitro is inhibited by 2–15 µM DFO in a dose-dependent manner, whereas the iron-saturated chelator does not affect it (Raventos-Suarez et al. 1982). These results were confirmed by our experiments. The 1,000-fold concentration (2 mM) was ineffective when the chelator was saturated with Fe$^{3+}$ (data not presented). Giemsa-stained preparations of *P. falciparum*-parasitized erythrocytes showed that the treatment of ring stages and early trophozoites with 50 µM DFO over 10 h resulted in death of the parasites at the late trophozoite stage (Fig. 1). Treatment with 1.7 mM DFO over 12 h gave the same effect. In both experiments, there was no visible effect on the parasites within the first 4.5 h. Using 14C-DFO, all supernatants contained almost the same amount of radioactivity (Table 1). The effectiveness of the washing procedures is reflected in the decreased amount of radioactivity observed in the supernatants. The ratio of radioactivity in the last supernatants from non-parasitized and par-