Enhanced expression of *Plasmodium falciparum* heat shock protein PFHSP70-I at higher temperatures and parasite survival

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(Received 22 September 1994; revision received 17 October 1994; accepted 19 October 1994)

Abstract: The effect of various body temperatures, encountered during malaria fever, on the synthesis of *Plasmodium falciparum* heat-shock protein called PFHSP70-I and parasite growth rates among five different isolates are described. The results show that after the exposure of parasites at 39°C for 30 min the amount of PFHSP70-I in all five isolates increased markedly and significantly, whereas parasite growth rates and the amount of total blood stage antigens remained almost unaffected. This indicates that the PFHSP70-I gene responds to heat-shock by producing higher amounts of PFHSP70-I protein, presumably to protect the parasite from being killed during malaria fever.

Key words: Human malaria; Parasite isolates; Fever; *Plasmodium falciparum*

Introduction

Almost all cellular organisms synthesize heat-shock proteins as a biochemical device to protect themselves against physical stress such as heat. These proteins play a wide variety of roles \([1,2]\). The human malaria parasite *Plasmodium falciparum* synthesizes several such proteins. Their genes are located on different chromosomes which are expressed independently, unlike *Trypanosoma* where these genes are arranged in tandem and transcribed as a polycistronic mRNA though translated separately \([3,4]\). Earlier we isolated one of the *P. falciparum* heat-shock protein ‘PFHSP70-I’ genes \([5]\). This protein is expressed in abundant quantities by various stages of the parasite. The PFHSP70-I has been proposed to be a candidate antigen for a malaria vaccine and immunodiagnostic \([6–10]\). However, its functional properties remain unclear \([11–14]\). In the present study we show that the amount of PFHSP 70-I increased at 39°C whereas parasite growth rates remained the same.
Materials and Methods

Parasite culture

Five different clinical isolates of *P. falciparum* were grown in larger volumes until the parasitemia reached 5%. The cultures, from each isolate, were then distributed in 4 tubes with equal number of parasites and exposed to 35, 37, 39 and 41°C for 30 min. After temperature shock, an aliquot from each tube was taken to determine the growth rate at 5% haematocrit and 1–1.5% parasitemia. Growth rates were determined after 96 h by examining Giemsa stained blood smears using the following formula:

\[
\text{No. of parasites per 5000 RBC after 96 h} \div \text{No. of parasites per 5000 RBC at 0 hour.}
\]

Antigen preparation

Recombinant PFHSP70-1 was prepared from cultures of the plasmid named HS4 where a cDNA coding for C-terminal half of the PFHSP70-I had been cloned into pBR322 by GC tailing [5]. The cultures were centrifuged and antigen was prepared by freezing and thawing the pellet, followed by brief sonication as described earlier [9]. After sonication the soluble extract was used for immunization. The crude blood stage *P. falciparum* antigen was prepared from the cultured infected RBCs. Parasites were made free from RBCs by 1% Triton X-100 and then pelleted by centrifugation at 3000 rpm for 20 min at 4°C. The pellet was washed 3 times with 0.15 M phosphate buffered saline (PBS, pH 7.2). Finally, the pellet was resuspended in PBS and then sonicated. The soluble extract was used for immunization and coating the microtiter plates for ELISA. Antibodies against recombinant PFHSP 70-I or crude blood stage antigen were raised in 6- to 8-week old BALB/c mice. They were immunized intraperitoneally with 100 µg protein mixed with complete Freund’s adjuvant. After 15 days a second injection of 100 µg protein was given with incomplete Freund’s adjuvant. The third dose was given after 15 days without any adjuvant. Seven days after third injection, antibody titre was determined using tail blood.

ELISA

The enzyme linked immunosorbent assay was performed using crude blood stage antigen from heat-shocked or non-heat-shocked parasites. Similarly, normal human (O + ) RBCs were also treated and used as a negative control. ELISA was performed as previously described in [9]. Briefly, each well of 96 microtiter plate received 10 µg protein. The antisera raised against crude blood stage antigen and PFHSP70-I were used at a 1:500 dilution. The second antibody was anti-mouse Ig conjugated to horseradish peroxidase (1:1000 dilution). The colour was developed by using a substrate, O-phenylene diamine/H2O2. The OD at 490 nm was monitored in a Titer track ELISA reader (Flow Lab., Irvine, Scotland).

Statistical analysis

A Randomized block ANOVA test was performed to evaluate the significant differences in the ELISA values of PFHSP70-I and total blood stage antigen, after the parasites were exposed to

<table>
<thead>
<tr>
<th>Name of isolate</th>
<th>Place of collection</th>
<th>Distance from Delhi (kilometers)</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDL-1</td>
<td>Rohini, Delhi</td>
<td>–</td>
<td>October, 1990</td>
</tr>
<tr>
<td>GP-30</td>
<td>Piyawali, Ghaziabad, UP</td>
<td>50</td>
<td>November, 1988</td>
</tr>
<tr>
<td>FSJ-A6</td>
<td>Shahjahanpur, UP</td>
<td>350</td>
<td>October, 1987</td>
</tr>
<tr>
<td>FSJ-B5</td>
<td>Shahjahanpur, UP</td>
<td>350</td>
<td>February, 1988</td>
</tr>
<tr>
<td>FJB-D2</td>
<td>Mandla, Jabalpur, MP</td>
<td>975</td>
<td>February, 1988</td>
</tr>
</tbody>
</table>

Blood samples were collected from *P. falciparum* infected patients who were running fever and were slide-positive. The infected RBCs were purified and adopted in culture. UP, Uttar Pradesh; MP, Madhya Pradesh.
Results and Discussion

The *P. falciparum* isolates were established in culture using infected blood obtained from patients who lived in various parts of India (Table 1). These isolates had different growth rates; FDL1 from Delhi grew at a maximum rate (Fig. 1 inset). The differences in the growth rates of these isolates were statistically significant from each other. Fig. 1 also shows that these growth rates remained distinct from each other when they were exposed to various temperatures. The optimum growth rates of these isolates were observed at 37°C (4.87 ± 0.73). These growth rates were reduced to 4.51 ± 0.67 (P < 0.05) if the cultures were exposed to 35°C for 30 min prior to normal growth. However, a drastic reduction in growth rates were observed after 41°C exposure, 2.37 ± 0.73 (P < 0.001). Interestingly, growth rates remained almost same, 4.88 ± 0.63 (P < 0.05), if cultures were exposed to 39°C.

The ELISA results using total parasite antigens (Fig. 1) showed significant differences between certain isolates (FDL1 vs FSJA6, P < 0.01; GP30 vs FSJA6, P < 0.05; GP30 vs FSJBS, P < 0.05; FSJA6 vs FJB D2, P < 0.05). But in case of the heat-shock protein PF HSP 70-I there was no significant difference among these isolates except that Jabalpur isolate FJBD2 showed slightly
higher ELISA values than Shahjahanpur isolate FSJ A6 (P < 0.05). The effect of various temperatures on the ELISA values of the total blood stage parasite antigens followed the same profile as that of the parasite growth rates (Fig. 1). The optimum values were observed at 37°C. These remained the same even if the parasites were exposed to 39°C. The ELISA values for total antigen were reduced at 35°C (P < 0.05) but the maximum reductions were observed at 41°C (P < 0.001). The effect of these temperatures on the ELISA values for heat-shock protein (PFHSP-70-I) were different from total parasite antigens, except, for 41°C where both showed a drastic reduction (P < 0.001). The ELISA values for PFHSP70-I were enhanced significantly following 39°C exposure (P < 0.001) as shown in Fig. 1. There was no significant change in the ELISA values for this protein at 35°C (P > 0.05).

The results obtained in the present study show that the expression of PFHSP70-I was elevated significantly, after exposure of the parasites to 39°C for 30 min (in comparison with 37°C) though the parasite growth rates and the expression of total blood stage antigens remained unaffected. In contrast, there was no effect on the expression of PFHSP70-I after 35°C exposure compared to 37°C, whereas the parasite growth rates and the total parasite antigen expression were reduced. These results indicate that the elevated temperatures induce the expression of PFHSP70-I. The enhanced expression of PFHSP70-I could be significant for survival of the parasite. However, it seems that PFHSP70-I can rescue the parasite only up to a certain extent i.e., 39°C. Beyond this limit, death may occur. At 41°C the parasite growth rate was adversely affected and so were the ELISA values for total blood stage parasite antigens and PFHSP70-I. It is speculated that the secondary and tertiary structures of the proteins could have been disrupted at this temperature (may be due to denaturation) because the total protein contents were kept constant for performing ELISA. In ELISA, the B-cell epitopes of an antigen need to be presented in a proper conformation for the respective antibody recognition.

It is concluded that the synthesis of PFHSP70-I is increased at 39°C to protect the parasite from being killed. At 41°C the amount of PFHSP70-I is reduced whereas the parasite death rate is increased. The results indicating a probable correlation between PFHSP70-I expression and parasite survival rates.

Acknowledgements

The financial assistance (to YDS) came from The Indian Council of Medical Research, The Department of Biotechnology (Government of India), The Council of Scientific and Industrial Research. We thank Dr. V.P. Sharma and Dr. Indira Nath for helpful discussions and Dr. S.N. Dwivedi and Dr. R.M. Pandey for statistical analysis.

References


