ABSTRACT

Malaria is caused by *Plasmodium* parasites and is the world's most devastating tropical infectious disease. The need for identifying novel drug targets is fuelled by an increased resistance of these parasites against available drugs. The human host red cell membrane plays an important role during invasion and subsequent development of the parasite within the red cell and undergoes several structural, functional and biochemical changes triggered by various protein-protein interactions between the parasite and the host cells. These interactions form a fundamental part of malaria research, since the parasite spends the pathogenic stage of its life cycle in the human erythrocyte. The *Plasmodium* kinome is complex and the exact role of protein phosphorylation in malaria parasites is not yet fully understood. This study aims to characterise the kinase domain of *Plasmodium falciparum* (3D7) Protein Kinase 8 (*Pf*PK8), described as a putative protein on the *Plasmodium falciparum* database. *Pf*PK8 is encoded by the *Pf*B0150c gene (recently renamed as PF3D7_0203100) situated on chromosome 2 of the parasite genome. A 1 507bp section of the *Pf*B0150c gene, containing a 822bp centrally located kinase domain was cloned into a pTriEx-3 expression vector. A soluble recombinant octa-histidine-tagged *Pf*PK8 was expressed in *Escherichia coli* Rosetta 2 (DE3) cells, but with relatively low yield and purity. To improve the expression, a recombinant *Pf*B0150c-baculovirus infected *Spodoptera frugiperda* (*Sf*9) insect cell system was attempted, but without success. A different tag was employed and glutathione-S-transferase-*Pf*PK8 was successfully expressed in *Escherichia coli* Rosetta 2 (DE3) cells, with a higher yield and purity. Recombinant GST-*Pf*PK8 was used in non-radioactive coupled spectrophotometric kinase assays in the presence of known kinase substrates casein, MBP and H1 to determine kinetic parameters of the enzyme. It phosphorylated all three substrates at a temperature of 37ºC and pH of 7.4. Recombinant GST-*Pf*PK8 was inactive at a pH below 6 and most active at pH 7.4. The relative activity of the enzyme was highest at a temperature synonymous to a fever spike in a *Plasmodium falciparum* infected individual. Secondary structural analysis of *Pf*PK8 revealed the position of a conserved substrate binding domain containing an ATP-binding site and binding loop within the kinase domain. The kinase domain of r*Pf*PK8 was modelled using available crystal structures of its identified homologues. The gene is expressed throughout the intraerythrocytic stages of the parasite life cycle, as well as in gametocytes. Protein-protein binding studies revealed that host-parasite protein-protein interactions exist between r*Pf*PK8 and erythrocyte membrane protein, band 3. *Plasmodium falciparum* PK8 could therefore

play a role during invasion of host erythrocytes and during the intraerythrocytic development of the parasite, by phosphorylating red blood cell membrane proteins. This study provides the groundwork for future X-ray crystallographic studies to elucidate the structure of the enzyme, and for additional gene manipulation experiments to ascertain whether it is essential for parasite survival in all the intraerythrocytic stages and therefore a potential new drug target candidate.