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The MTIP–Myosin A Complex in Blood Stage Malaria Parasites

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Parasites of the Apicomplexa phylum use an actomyosin motor to drive invasion of host cells. The motor complex is located at the parasite's periphery between the plasma membrane and an inner membrane complex. A crucial component of this complex is myosin tail domain interacting protein (MTIP) identified in the murine malaria parasite *Plasmodium yoelii*. Here, we show that MTIP is expressed in *Plasmodium falciparum* merozoites, localises to the periphery of the cell and is present in a complex with myosin A. The MTIP–myosin A tail interaction has a K_d of 235 nM and calcium ions do not play a role in modulating the binding affinity of the two molecules, despite reports of a predicted EF-hand in MTIP. Antibodies to MTIP were used to immobilise the MTIP–myosin A complex, allowing actin binding and motility to be examined. Measurement of actin filament velocities powered by myosin A revealed a velocity of 3.51 μm s^{-1}, a speed comparable to fast muscle myosins. A short peptide derived from the tail of myosin A (C-MyoA) bound to MTIP and was able to disrupt the association of MTIP and myosin A in parasite lysates. C-MyoA peptidomimetic compounds that disrupt the MTIP–myosin A interaction are predicted to inhibit parasite motility and host cell invasion, which may be targets for new therapeutic approaches.

Keywords: Plasmodium; malaria; invasion; myosin; motility

Introduction

In the life-cycle of the parasite that causes malaria, *Plasmodium* spp., there are three invasive stages that must enter erythrocytes and hepatocytes of the vertebrate host and cross the gut wall of the mosquito host. The force required for invasion of the host cell by the parasite is generated by an actomyosin motor. Compounds that destabilise actin filaments such as cytochalasin B have been shown to inhibit invasion of erythrocytes by *Plasmodium* merozoites,[1] as have those that block the ATPase activity of myosin.[2] The components of the actomyosin motor complex providing the necessary propulsion are thought to be the same for all invasive stages of *Plasmodium*: the merozoite, sporozoite and ookinete (reviewed by Fowler *et al*.[3]). Myosin A, a type XIV myosin with a characteristically short tail,[4,5] has been suggested to be responsible for providing the power for invasion and has been localised to the periphery of *Plasmodium* merozoites[2] and sporozoites.[6] *Plasmodium* zoite stages possess an inner membrane complex (IMC) lying just beneath the plasma membrane. This IMC consists of a double membrane that is continuous around the entire cell, except at the apical end of the parasite.[7] In the model suggested by Bergman *et al*., myosin A is linked to the IMC by its light chain, myosin tail domain interacting protein (MTIP).[6] This linkage has been proposed to provide a stable anchor for myosin and may, like other myosins, also contribute to the so-called lever arm of the motor. Whether MTIP achieves its anchoring directly or *via* interaction with an intermediary protein is not known, although recently it has been shown that *Toxoplasma gondii* myosin light chain (MLC) is present in a complex with TgMyosin A and two further proteins, TgGAP45 and TgGAP50.[8] MTIP

Abbreviations used: MTIP, myosin tail domain interacting protein.

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has homologues throughout *Apicomplexa*, and has been suggested to possess at least one EF-hand,[6,9] the most common calcium-binding motif of proteins.[10] The location of the actomyosin motor between the IMC and plasma membrane of the invasive zoite has led to speculation on the topology of components of the motor itself and also the presence of proteins that link the motor to surface adhesins of the parasite. In *P. falciparum* sporozoites it has been shown that aldolase links the cytoplasmic tail of the surface adhesin TRAP (thrombospondin-related anonymous protein) to actin, thus providing a link from the host cell surface to the actomyosin motor *via* parasite surface proteins.[11]

Here, we aim to further examine the complex formed between MTIP and myosin A, and to also test the ability of parasite-derived myosin A to translocate actin. Using spectroscopic methods we demonstrate and quantify the high-affinity interaction between recombinant MTIP and a peptide corresponding to the C terminus of myosin A. In order to show that the MTIP–myosin A complex works as a molecular motor that will drive the movement of actin in an ATP-dependent fashion we used an *in vitro* motility assay. We have found that myosin A immobilised specifically by an anti-MTIP antibody translocates actin filaments at 3.51 μm s^{-1}.[1] This velocity is consistent with that of *T. gondii* myosin A[9] and within the range required to drive invasion of erythrocytes by merozoites at the speeds observed.[12]

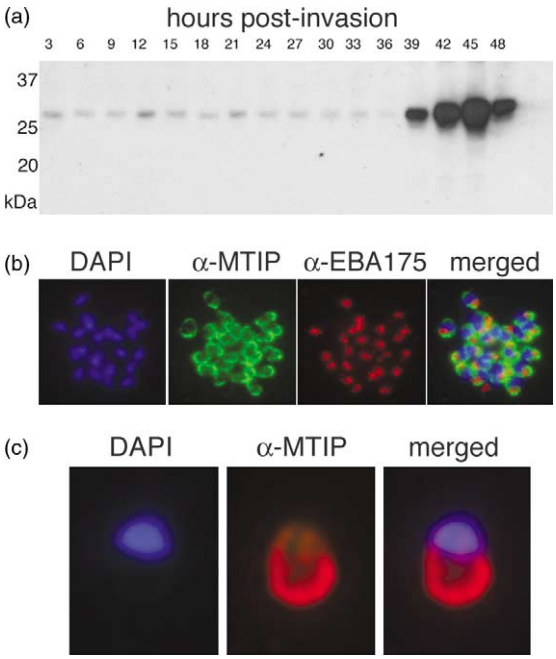

Figure 1. Expression and localisation of MTIP. (a) Western blotting with MTIP-specific antibody of *P. falciparum* proteins extracted from parasitised cells at the indicated time after invasion of erythrocytes. MTIP expression peaks at 42–45 h after invasion. The mobility of molecular mass markers (kDa) is shown on the left of the panel. (b) Indirect immunofluorescence using an antibody against MTIP demonstrates that MTIP (green) is located at the periphery of each merozoite. The nuclei are counterstained with DAPI (blue) and for reference an apical protein, EBA175, is shown stained in red. The merged image is shown on the right. (c) MTIP (red) concentrated at the anterior end of a free merozoite counterstained with DAPI to show the nucleus (blue).

Results

Localisation and timing of MTIP expression

Following erythrocyte invasion by merozoites the parasite develops over a 48 h period, forming a multinucleated schizont that leads to release of new merozoites and re-invasion. Protein was extracted from synchronised parasite cultures at 3 h intervals after invasion of erythrocytes. An antibody raised against recombinant MTIP specifically recognised a 27 kDa protein by Western blot analysis. The protein is present throughout the cycle, with the peak of expression occurring at 45 h, just before re-invasion (Figure 1(a)). Using the same antibody to locate MTIP in late schizonts we observed staining around the periphery of each merozoite within the schizont (Figure 1(b)). This is similar to the previous localisation of MTIP to the inner membrane complex within sporozoites of *P. yoelii*.[6] In free merozoites MTIP has an asymmetric distribution around the cell's periphery (Figure 1(c)). There is strong fluorescence at the apical end of the parasite with a much-reduced signal at the basal end (distinguished by the presence of the nucleus). Immunoprecipitation from parasite lysates with MTIP-specific antibody revealed the presence of a co-precipitating ~95 kDa protein (Figure 2, left panel). A Western blot of this immunoprecipitated complex with a myosin A-specific antibody confirmed the identity of the protein as myosin A (Figure 2, right panel).

Binding of MTIP and myosin A

The interaction between MTIP and myosin A has been attributed to the binding of the C terminus of myosin A to MTIP.[6] We used a 17 amino acid residue peptide to represent the C terminus of the myosin A tail (designated C-MyoA) in order to study the interaction with MTIP. MTIP was expressed in *Escherichia coli* and purified to homogeneity by immobilised-metal affinity chromatography (Figure 3(a), left). The far-UV CD spectrum of MTIP (Figure 3(a)) is characteristic of a protein with a high α-helical content. Analysis using standard calculation methods (see Materials and Methods) gave 38% α-helix, 13% β-sheet, 21% turn, and 28% random coil. The spectrum is independent of pH over the range 6.5–8.0 and of concentration over the range 2–30 μM. The inset to Figure 3(a) shows that there is a small but reproducible effect of