Structure and Characterisation of a Key Epitope in the Conserved C-Terminal Domain of the Malaria Vaccine Candidate MSP2

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Abstract

Merozoite surface protein 2 (MSP2) is an intrinsically disordered antigen that is abundant on the surface of the malaria parasite *Plasmodium falciparum*. The two allelic families of MSP2, 3D7 and FC27, differ in their central variable regions, which are flanked by highly conserved C-terminal and N-terminal regions. In a vaccine trial, full-length 3D7 MSP2 induced a strain-specific protective immune response despite the detectable presence of conserved region antibodies. This work focuses on the conserved C-terminal region of MSP2, which includes the only disulphide bond in the protein and encompasses key epitopes recognised by the mouse monoclonal antibodies 4D11 and 9H4. Although the 4D11 and 9H4 epitopes are overlapping, immunofluorescence assays have shown that the mouse monoclonal antibody 4D11 binds to MSP2 on the merozoite surface with a much stronger signal than 9H4. Understanding the structural basis for this antigenic difference between these antibodies will help direct the design of a broad-spectrum and MSP2-based malaria vaccine. 4D11 and 9H4 were reengineered into antibody fragments [variable region fragment (Fv) and single-chain Fv (scFv)] and were validated as suitable models for their full-sized IgG counterparts by surface plasmon resonance and isothermal titration calorimetry. An alanine scan of the 13-residue epitope 3D7-MSP2$_{207–222}$ identified the minimal binding epitope of 4D11 and the key residues involved in binding. A 2.2-Å crystal structure of 4D11 Fv bound to the eight-residue epitope NKENCGAA provided valuable insight into the possible conformation of the C-terminal region of MSP2 on the parasite. This work underpins continued efforts to optimise recombinant MSP2 constructs for evaluation as potential vaccine candidates.

Introduction

Development of a malaria vaccine is a high priority. The mosquito-borne disease, caused by protozoa of the *Plasmodium* genus, is responsible for 200 million cases and over 430,000 deaths per year [1]. Although vector control and drug interventions have significantly decreased mortality over the past decade, the global eradication of malaria calls for a robust and effective vaccine [2]. The most advanced vaccine candidate is RTS,S/AS01, a pre-erythrocytic vaccine that has shown only modest efficacy in young children and infants in Phase III trials [3]. Blood-stage vaccines are aimed at inhibiting parasite growth and proliferation,

thereby reducing the severity of symptoms and in turn mortality [4,5]. The eradication of malaria will require the development of multistage vaccines able to prevent blood-stage infection and block transmission.

Merozoite surface protein 2 (MSP2) is a ~23-kDa glycosylphosphatidyl inositol (GPI)-anchored protein present in the asexual blood stages of *Plasmodium falciparum* and is one of the most abundant proteins on the surface of the merozoite [6,7]. The protein is highly polymorphic, particularly in the central variable region, which consists of tandemly arrayed repeat sequences and dimorphic sequences that differentiate the protein into two allelic families, 3D7 and FC27 (Fig. 1a) [8,9]. The central variable region is flanked by a highly conserved 25-residue N-terminal region and ~50-residue C-terminal region, the latter containing the only disulphide bond of the protein. Similar to many other blood-stage antigens [10–12], MSP2 is an intrinsically disordered protein lacking a well-defined structure in solution [13], although the protein has been found to have a propensity for amyloid-like fibril formation [14,15]. The structural characteristics of MSP2 when interacting with the merozoite surface and those mediated by the GPI-anchor are still being explored. NMR studies have shown that the conserved N-terminal region adopts an α-helical conformation when in the presence of lipid [16] and identified some motional restriction in the conserved C-terminal region caused by the single disulphide bond [13].

MSP2 is well characterised as a vaccine candidate and has been included in several human vaccine trials [17–21]. In Phase I-IIb trials, recombinant 3D7-MSP2 in combination with MSP1 and RESA was tested in Papua New Guinean children [18,22]. A 62% reduction in parasite densities was observed, although this was biased towards parasites expressing the 3D7 allele of MSP2 that was used in the vaccine [19]. This implies that the response to MSP2 dominated the protective effect in this trial, but the MSP2 response was highly strain-specific. A vaccine containing recombinant forms of both 3D7 and FC27 MSP2 was tested in Phase I trials and induced antibodies against both alleles that were able to inhibit parasite growth by antibody-dependent cellular inhibition [20] and complement-mediated inhibition [23]. A recent approach to circumvent the problems of strain-specific immune responses involved the use of MSP2 chimeras that incorporated conserved and variable regions of the 3D7 and FC27 alleles [24]. In mice, these chimeras were able to induce a robust anti-MSP2 antibody response across both alleles. Nonetheless, the success of an MSP2 vaccine is likely to be significantly enhanced by a protective immune response targeting conserved epitopes.

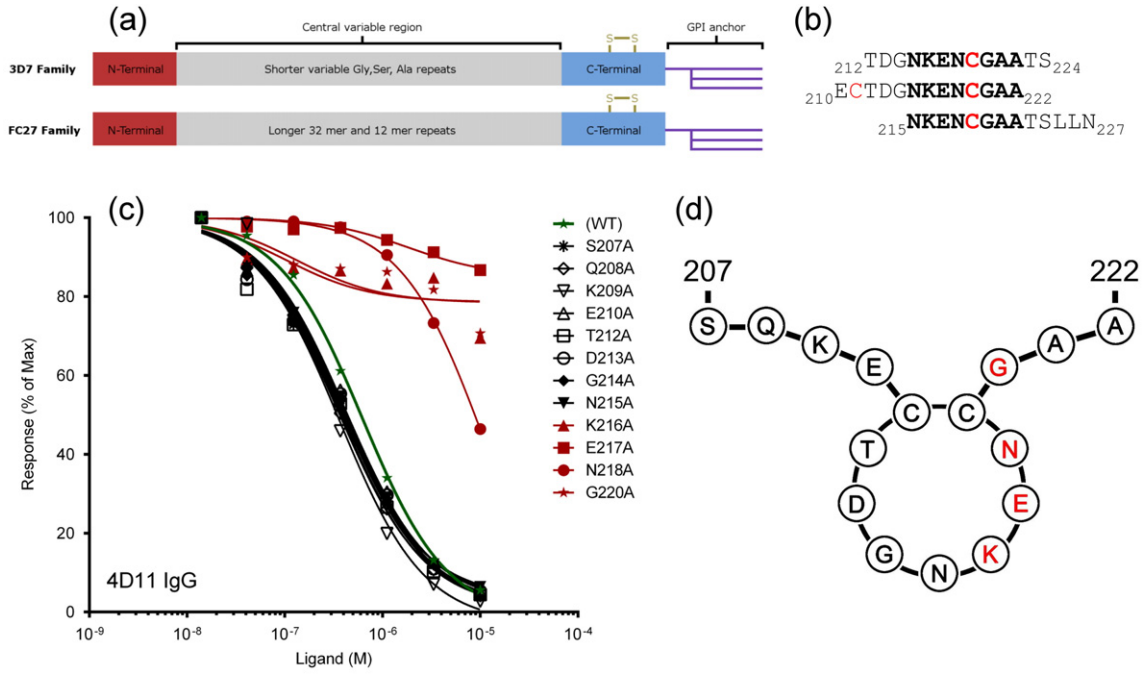

Fig. 1. (a) Schematic of the primary structures of 3D7 and FC27 MSP2. (b) Aligned 13-residue peptides from peptide array able to bind with mAb 4D11. 3D7-MSP2$_{212–224}$ and 3D7-MSP2$_{210–222}$ reacted strongly with mAb 4D11 by ELISA [25]. Overlapping residues are shown in bold, cysteine residues are shown in red. (c) SPR competition assay of alanine scan 16-residue peptides of 3D7-MSP2$_{207–222}$. Black lines show alanine mutants with no effect on binding, the green line indicates the wild-type binding of 3D7-MSP2$_{207–222}$, and red lines indicate the alanine mutations that decreased the binding to 4D11 IgG. (d) Schematic representation of disulphide-bonded peptide sequence indicating the location of key residues in red.