Biochimica et Biophysica Acta 1818 (2012) 2572–2578

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Lipid interactions of the malaria antigen merozoite surface protein 2

Christopher A. MacRaild [a,1], Marie Ø. Pedersen [a,1], Robin F. Anders [b], Raymond S. Norton [a,*]

[a] Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville 3052, Australia
[b] Department of Biochemistry, La Trobe University, Victoria 3086, Australia

ARTICLE INFO

Article history:
Received 23 April 2012
Received in revised form 20 June 2012
Accepted 21 June 2012
Available online 27 June 2012

Keywords:
Malaria
Plasmodium falciparum
Merozoite surface protein 2
Structure
NMR

ABSTRACT

With more than half the world's population living at risk of malaria infection, there is a strong demand for the development of an effective malaria vaccine. One promising vaccine candidate is merozoite surface protein 2 (MSP2), which is among the most abundant antigens of the blood stage of the *Plasmodium falciparum* parasite. In solution, MSP2 is intrinsically unstructured, but little is known about the conformation of native MSP2, which is GPI-anchored to the merozoite surface, or of the implications of that conformation for the immune response induced by MSP2. Initial NMR studies have shown that MSP2 interacts with lipid micelles through a highly conserved N-terminal domain. We have further developed these findings by investigating how different lipid environments affect the protein structure. All of the tested lipid preparations perturbed only the N-terminal part of MSP2. In DPC micelles this region adopts an α-helical structure which we have characterized in detail. Our findings suggest a possible mechanism by which lipid interactions might modulate immune recognition of the conserved N-terminus of MSP2, potentially explaining the apparent immunodominance of the central variable region of this important malaria antigen.

1. Introduction

Antigens on the surface of the *Plasmodium falciparum* merozoite represent attractive candidates for inclusion in a malaria vaccine [1]. Merozoite surface protein 2 (MSP2) is a ~28 kDa protein anchored to the merozoite membrane by a C-terminal glycosylphosphatidylinositol (GPI) moiety [2] and is one of the most abundant proteins on the surface of the merozoite [3]. Because of its localisation at the merozoite surface, MSP2 is directly accessible to immune recognition by plasma antibodies. People naturally-exposed to malaria acquire high levels of anti-MSP2 antibodies and these responses have been associated with protection from symptomatic disease [4–9]. For these reasons, vaccines incorporating MSP2 are under active development [1,10,11]. Recent clinical trials of MSP2-based vaccines have established their safety and partial efficacy, but have highlighted the need to induce a response that better matches the specificity of the naturally acquired response [11,12].

MSP2 exists as one of two major allelic types, FC27 and 3D7, which are distinguished by the sequences of a highly polymorphic central region that consists of non-repeat sequences surrounding tandem repeat sequences [13,14]. FC27 forms of MSP2 contain 32-residue and 12-residue repeats, whereas 3D7 forms of the antigen contain much shorter repeats. The diversity of the central regions is likely to have evolved under immune pressure from the human host [14]. All MSP2 alleles have conserved 25-residue N-terminal and ~50-residue C-terminal amino acid sequences that flank the central variable region (Fig. 1, Supplementary Figure S1). An MSP2 construct has been expressed in *E. coli* where the central variable region was deleted, leaving the N- and C-terminal domains joined directly. This recombinant construct is immunogenic, but the antibodies raised have a different specificity from those in naturally-infected hosts, and the protein was poorly recognised by sera from malaria-exposed humans that are reactive against native MSP2 [15]. These results suggest that the N- and C- terminal regions of MSP2 are not exposed to the human immune system in the course of a natural malaria infection. Recently, we have characterised a panel of monoclonal antibodies raised against recombinant MSP2. Of this panel, most of those that recognise the conserved regions of recombinant MSP2 either fail to recognise, or react poorly with, MSP2 on the parasite surface, whereas those that recognise the central variable region of MSP2 react well with the parasite surface [16].

Recombinant MSP2 is intrinsically unstructured, with a high propensity for fibril formation [17,18]. Like many malaria surface antigens, MSP2 is GPI anchored to the merozoite surface. However, the structural characteristics of MSP2 on the merozoite surface remain unknown. GPI-mediated lipid interactions alter the antigenic properties of proteins of other parasitic organisms [19], but these issues have not been addressed in the case of MSP2 or other malaria antigens. Recently, we have shown that the conserved N-terminal region of MSP2 interacts with dodecyl phosphocholine (DPC) micelles, in the

Abbreviations: MSP2, merozoite surface protein 2; DPC, dodecyl phosphocholine; LPPG, lyso-palmatoyl phosphatidylglycerol; DLPC, dilauryl phosphatidylcholine; SUVs, small unilamellar vesicles

* Corresponding author. Tel.: +61 3 9903 9167; fax: +61 3 9903 9582.
E-mail address: ray.norton@monash.edu (R.S. Norton).

[1] Contributed equally.

0005-2736/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2012.06.015

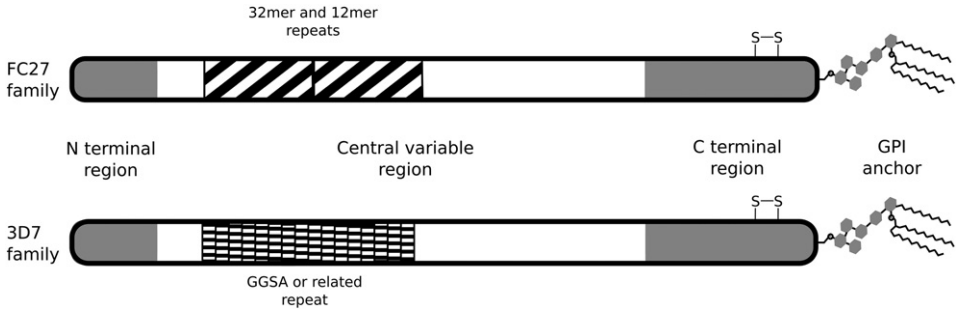

Fig. 1. Schematic of the two families of MSP2, FC27 and 3D7.

absence of the GPI anchor [18]. This raises the possibility that lipid interactions may play a role in determining the apparent immunodominance of the central variable region of MSP2. Here we undertake a detailed structural characterisation of the interaction of MSP2 with lipid surfaces, in order to understand the implications for the interactions of MSP2 with the host immune system and with a view to designing more effective MSP2-based malaria vaccines.

2. Experimental procedures

2.1. Materials

All studies described here were carried out with the recombinant FC27 allelic form of MSP2 expressed in *E. coli*. Untagged full-length FC27 MSP2 was expressed and purified using a strategy specific for recombinantly expressed unstructured proteins, as described previously [18]. Truncated FC27 MSP2 ($MSP2_{1\text{-}25}$) was either expressed and purified according to the protocol by Low et al. [20] or purchased as a synthetic peptide from GL Biochem (Shanghai, China). ^{15}N-labelled full-length or truncated MSP2 was prepared by growing expression cultures in M9 minimal medium, with 1 g/L ^{15}N ammonium chloride as the sole nitrogen source.

2.2. Sample preparation

All MSP2 samples used in titration experiments were freshly prepared by dissolution of freeze-dried MSP2 in the appropriate buffer and briefly heated to 95 °C to dissolve any aggregates present. Working stocks of $^{2}H_{38}$-DPC (Cambridge Isotope Laboratories) and LPPG (Avanti Polar Lipids) were freshly prepared in the appropriate buffer. Lipid SUVs were prepared by suspending dry DLPC (Avanti Polar Lipids) in buffer and subjecting the mixture to probe sonication and vortex mixing until a stable transparent solution was achieved. $MSP2_{1-25}$ samples for structure determination were prepared by dissolution of freeze-dried peptide directly in the buffered DPC solution (50 mM DPC, 20 mM sodium acetate, pH 4.5).

2.3. NMR spectroscopy

Samples for NMR spectroscopy contained 5% $^{2}H_2O$ and 0.01% dioxane, the latter as an internal reference for chemical shift and diffusion coefficient measurements. NMR spectra were acquired on Bruker Avance 500 and 600 MHz spectrometers equipped with cryoprobes, and a Bruker DRX-600 spectrometer with a room-temperature probe. ^{1}H chemical shifts were referenced with dioxane set as 3.751 ppm, and ^{15}N and ^{13}C chemical shifts were referenced indirectly from ^{1}H using the ratios $\gamma_N/\gamma_H = 0.101329118$ and $\gamma_C/\gamma_H = 0.251449453$ [21]. Two-dimensional ^{1}H-^{15}N HSQC spectra were typically acquired with 2048 (^{1}H) and 256 (^{15}N) points and spectral widths of 9 ppm (^{1}H) and 24 ppm (^{15}N). Diffusion measurements were performed using a pulsed field gradient longitudinal eddy-current delay pulse sequence, as implemented by Yao et al. [22]; a series of 12 spectra was acquired with the strength of the diffusion gradient varying from 3.44 G cm^{-1} to 36.22 G cm^{-1}. NMR Spectra were processed using Bruker Topspin or NMRpipe [23], and assignment of $MSP2_{1-25}$ was done in SPARKY [24].

2.4. Structure calculation

A structural ensemble of $MSP2_{1\text{-}25}$ was calculated based on NOE restraints derived from the 2D NOESY spectrum. Calibration of NOE distance restraints and initial structure calculations were carried out using CYANA (version 1.0.6). φ-angles were restricted to negative values ($-90° \pm 90°$) [25], and, for residues shown by chemical shift and NOE patterns to be in the α-helical region of $MSP2_{1-25}$, the φ and ψ angles were restricted further to $-64° \pm 30$ and $-40° \pm 30°$, respectively. Final structure calculations were performed in NIH-XPLOR [26], with 100 conformers calculated with high temperature simulated annealing in torsion angle space followed by extensive Cartesian annealing and refinement. The final ensemble of 20 structures was selected on the basis of overall XPLOR energy, with the exclusion of structures with an NOE energy term exceeding 10 kcal/mol; this later filter being required to exclude a small number of low-energy structures with anomalously high NOE energy due to an obvious distortion around residue 15 in violation of several short-range NOEs (Table 1). The structure was validated using PROCHECK [27], with RMSD values and angular order parameters calculated using MOLMOL [28], and other structural analysis using PyMOL [29].

3. Results

3.1. MSP2–micelle interactions

We have previously shown that under acidic conditions, recombinant MSP2 lacking the C-terminal GPI anchor, interacts with zwitterionic DPC micelles, despite its marked hydrophilic character [18]. To further characterise this interaction, we performed DPC titrations under acidic (pH 3.5 and 4.5) and neutral (pH 7.3) conditions, using ^{1}H– ^{15}N HSQC spectra of MSP2 to monitor the interaction. Under all of the conditions tested we saw no change to MSP2 spectra in the presence of DPC below its critical micelle concentration (CMC) of ~1 mM, indicating that MSP2 does not interact with isolated molecules of DPC (data not shown). In contrast, we observed significant changes at micellar concentrations as MSP2 interacted with the DPC micelle. This interaction caused substantial line-broadening and chemical shift perturbation involving the entire conserved N terminal region, and a few residues at the extreme C-terminus. No changes in either chemical shift or line-shape were observed for residues in the central variable region, or in most of the C-terminal region, indicating that these parts of MSP2 do not interact with the DPC micelle and are not significantly perturbed by the interaction of the N-terminus. The lack of line-broadening in these regions is consistent with the highly flexible nature of MSP2 [18], and indicates that these residues are conformationally decoupled from the DPC micelle, even when the N-terminal part of the molecule is bound.