NMR assignment of the Immune Mapped Protein 1 homologue (IMP1) in

Plasmodium falciparum

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Abbreviations

IMP1	Immune Mapped Protein 1
IPTG	Isopropyl -D-thiogalactopyranoside
LIC	Ligation independent cloning
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser effect enhancement spectroscopy
TOCSY	Total correlation spectroscopy

Abstract

Plasmodium falciparum is responsible for causing cerebral malaria in humans. IMP1 is an immunogenic protein, present in the parasite, which has been shown to induce an immune response against apicomplexan parasites in a species-specific manner. Here, we report the complete NMR assignments of PfIMP1.

Key word

IMP1, antigenic protein, *Plasmodium falciparum*, NMR

Background

Immune mapped protein 1 (IMP1) is an antigenic protein that is conserved across most apicomplexan parasites (Blake et al, 2011). It was first identified in *Eimeria maxima* and was found to raise protective immune response in hosts against the parasite (Blake et al, 2011). Cui X et al (2012) have shown that mice immunised with a DNA vaccine of *Toxoplasma gondii* IMP1 (TgIMP1) have a prolonged lifespan in comparison to control mice upon infection with *T. gondii*. Studies by Yin G et al (2013) have also shown that a chimeric subunit vaccine developed using truncated IMP1 from *Eimeria tenella* (EtIMP1) and a molecular adjuvant raises protective immunity against *E. tenella* infection in chickens. These studies show that IMP1 raises an antigenic response in hosts against parasites in a species specific manner.

The localisation of IMP1 within apicomplexan parasites is ambiguous. Cui X et al (2012) have shown that TgIMP1 is localised to the parasite membrane. Yin G et al (2013) suggest that EtIMP1 could be a membrane protein based on localisation studies and bioinformatics analysis. Although the function and localisation of IMP1 within the parasite is unknown, the antigenic response raised by the protein in hosts makes it an interesting candidate for structural studies. Hence, we perform an NMR analysis on an IMP1-like protein from *P. falciparum* (PfIMP1) to understand the three dimensional structure of the protein in solution. Here, we present the complete NMR assignments for IMP1 for PfIMP1.

Materials and Methods

PfIMP1 sequence (Accession number: XP_001349200) was synthesised following codon optimisation for recombinant expression in *E. coli*. The codon optimised PfIMP1 sequence was then amplified using primer set PfIMP1-NTH-F (5' -

TACTTCCAATCCATGAGCGAAGAAAAAGGT - 3') and PfIMP1-NTH-R (5' – TATCCACCTTTACTGTCAAAAGCTAATGCT - 3'). The PCR product was purified and cloned into pET28-NTH vector by LIC cloning. The resulting plasmid pET28-NTH-PfIMP1 was transformed into Rosetta2 cells (NEB, UK). The recombinant strains were grown in M9 minimal media containing 0.07% $^{15}\text{NH}_4\text{Cl}$ and 0.2% $^{13}\text{C}_6\text{-glucose}$ (Sigma) for $^{15}\text{N}/^{13}\text{C}$ -labeling of PfIMP1. Once $\text{OD}_{600} = 0.6$, expression was induced using 1mM IPTG. Following overnight incubation at 18°C, the cells were harvested by centrifugation at 4500 rpm for 15 minutes. The cells were lysed by sonication and the lysate was clarified by centrifugation at 16000 rpm for 40 minutes. The clarified lysate was purified by affinity chromatography using pre-packed Nickel column, 5 ml HisTrap FF (GE Healthcare, UK) in the AKTApurifier (GE Healthcare, UK). The purified protein was cleaved with TEV protease to remove the N-terminal His tag and further purified by Gel filtration using a Superdex 75 HiLoad 16/600 column (GE Healthcare, UK) with AKTApurifier (GE Healthcare, UK). The purified protein was then concentrated and dialysed into 50 mM Hepes, 250 mM NaCl and 10mM DTT, pH7.5. The final construct that was used to record NMR experiments contained a serine residue left from the TEV cleavage, followed by the complete amino acid sequence of PfIMP1. The molecular weight of this protein is 17.89 kDa. There are 159 residues in this construct but the methionine following the first serine residue is labelled as residue 1 throughout this article.

NMR spectra were recorded at 303K on Bruker DRX600 and DRX800 spectrometers equipped with cryo-probes. The Chemical shifts of ^1HN , ^{15}N , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$ and ^{13}CO cross peaks were assigned using CBCA(CO)NH, HNCACB, HNCO and HN(CA)CO. The aliphatic and aromatic side chain ^1H and ^{13}C assignments were obtained by using HBHA(CBCACO)NH, HCCH-TOCSY, (H)CC(CO)NH-TOCSY and $^1\text{H-C}^{13}\text{NOESY-HMQC}$ spectra.

NMR Assignments for PfIMP1

Backbone assignment of PfIMP1 was initially performed semi-automatically using MARS (Jung and Zweckstetter, 2004) then subsequently confirmed and completed manually. Due to the high number of lysines (13%) present in the PfIMP1 sequence, it was challenging to assign them as these side chain resonances were heavily overlapped. In total, ~96 % of all possible backbone atoms were assigned. Figure 1 shows the assigned ^{15}N , ^1H – HSQC spectrum. ~94% of the amino acid side chain atoms have also been assigned. The secondary structure prediction (Figure 2) was obtained from Talos N (Shen and Bax, 2013). The amide proton of I96 resonates at an unusual upfield-shifted position compared to the other structured amides. It is located in a loop region between secondary structure elements and is immediately preceded by an FP sequence. The proline residue within this loop likely configures the backbone conformation such that amide proton of I96 experiences a significant shielding ring current effect from F94.

References

- BLAKE, D., BILLINGTON, K., COPESTAKE, S. & OAKES, R. 2011. Genetic mapping identifies novel highly protective antigens for an apicomplexan parasite. *PLoS pathogens* 7.
- CUI, X., LEI, T., YANG, D., HAO, P., LI, B. & LIU, Q. 2012. *Toxoplasma gondii* immune mapped protein – 1 (TgIMP1) is a novel vaccine candidate against toxoplasmosis. *Vaccine*, 30, 2282-2287.
- JUNG, Y. S. & ZWECKSTETTER, M. 2004. Mars -- robust automatic backbone assignment of proteins. *J Biomol NMR*, 30, 11-23.
- YIN, G., QIN, M., LIU, X., SUO, J., TANG, X., TAO, G., HAN, Q., SUO, X. & WU, W. 2013. An Eimeria vaccine candidate based on Eimeria tenella immune mapped protein 1 and the TLR-5 agonist Salmonella typhimurium FliC flagellin. *Biochem Biophys Res Commun*, 440, 437-42.
- SHEN, Y. & BAX, A. 2013. Protein backbone and sidechain torsion angles predicted from NMR chemical shifts using artificial neural networks. *J Biomol NMR*, 56, 227-41.

Figure Captions

Fig.1 Assigned ^{15}N , ^1H – HSQC spectrum of recombinant PfIMP1. The peaks are labelled with single letter amino acid code followed by their position in the recombinant PfIMP1 sequence. The underlined residues indicate aliased amide signals (namely G82, G51 and G149).

Fig. 2 Secondary structure determination using TALOS-N (Shen and Bax, 2013). The predicted secondary structure of this protein consists of four α -helices and eleven β strands. Helices are located between residues 43-49, 58-80, 88-92 and 142-151. β strands are located between residues 5-12, 17-24, 32-36, 53-57, 83-86, 96-104, 107-112, 115-118, 124-128, 139-140 and 154-157.

Figure 1

Figure 2