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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of domain III of the ectodomain of apical membrane antigen 1 from *Plasmodium falciparum*

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Biological context

Apical membrane antigen 1 (AMA1) of the malaria parasite *Plasmodium falciparum* is expressed late in the development of the asexual blood stages of *P. falciparum* and other malaria parasites (Crewther et al., 1990). Although its function is unknown, AMA1 is released from the apical organelles onto the merozoite surface and is believed to play a role in parasite invasion. As immunisation with AMA1 provides protection in animal models of the human disease, it is a promising vaccine candidate (Good et al., 1998), which is currently undergoing early clinical trials.

The protective immune response to AMA1 is dependent on the disulphide bond stabilised structure of the ectodomain, as the antigen is not an effective vaccine after reduction and alkylation (Anders et al., 1998). The 16 conserved cysteine residues form eight intramolecular disulphide bonds, the arrangement of which suggests a three-domain structure, with domains I, II and III containing three, two and three disulphide bonds, respectively. Only six of the eight disulphide bonds have been assigned unequivocally (Hodder et al., 1996), the two unassigned disulphide bond connectivities being located in domain III. *Pf* AMA1 domain III comprises 110 residues, corresponding to residues 436–545 of the ectodomain. The construct examined in this study includes 12 N-terminal vector-encoded residues to give a total molecular mass of 14.3 kDa. The structure of this construct will define the disulphide connectivities in domain III and provide a molecular basis for interpreting the effects on antigenicity and immunogenicity of natural variations in the AMA1 sequence among different strains of *P. falciparum*.

Methods and experiments

Pf AMA1 domain III, uniformly labelled with ^{13}C and/or ^{15}N, was prepared by growing *E. coli* JPA101 strain in minimal media supplemented with $^{13}CH_3{}^{13}COONa$ as the sole carbon source and $^{15}NH_4Cl$ as the nitrogen source. The *E. coli* cell pellet was solubilised in 6 M guanidine-HCl, pH 8.0, and, after clarification by centrifugation, the supernatant was incubated in batch mode for 2 h with Ni-nitrilotriacetic acid (NTA) resin (Qiagen GmbH, Hilden, Germany). The eluted protein was refolded by dilution into a buffer containing 1 mM reduced glutathione and 0.25 mM oxidised glutathione, as previously described for the refolding of the AMA1 ectodomain (Anders et al., 1998). The refolded protein was further purified by anion-exchange chromatography followed by reversed-phase HPLC. The final product was a single peak on analytical reversed-phase HPLC, with $>$ 98% purity determined on SDS-PAGE.

Samples for NMR were prepared by dissolving lyophilised protein in 90% H_2O/10% $^{2}H_2O$ solution containing 0.05% NaN_3 but no buffer. Sample concentrations were $\sim$1 mM for both ^{15}N-labelled and double-labelled material and the pH was 3.4. In general, NMR spectra were recorded at 30 °C on Bruker AMX-500 and DRX-600 spectrometers

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equipped with triple-resonance probes. Additional spectra were recorded at 10 °C (pH 3.4) and pH 2.8 (30 °C) to assist with resonance assignment. NMR spectra recorded on the ^{15}N-labelled sample for backbone and side-chain resonance assignments included 3D ^{15}N-edited NOESY-HSQC (mixing time 150 ms), 3D ^{15}N-edited TOCSY-HSQC (isotropic mixing time 50 ms) and 2D ^{1}H-^{15}N HSQC (Cavanagh et al., 1996). Triple-resonance experiments recorded on the double-labelled sample included 3D HNCA, HNCO, CBCA(CO)NH, HCCH-TOCSY and CCCONH (Cavanagh et al., 1996; Sattler et al., 1999). Spectra were processed using XWINNMR (Bruker AG) and analysed using XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

Resonance assignments were made primarily from ^{15}N-edited NOESY-HSQC and TOCSY-HSQC spectra, together with data from HNCA, CBCA(CO)NH, and CCCONH experiments. This provided the ^{1}H and ^{15}N assignments, as well as the C^{α} and C^{β} resonance assignments and some C^{γ} side-chain assignments. Additional ^{1}H and ^{13}C assignments were obtained from the HCCH-TOCSY spectrum. Figure 1 shows a 2D {^{1}H,^{15}N} HSQC spectrum recorded on ^{15}N-labelled domain III. Minor peaks of weak intensity present in this spectrum are probably impurities, but could also arise from minor conformers associated with *cis-trans* isomerism of a proline residue in the largely unstructured loop. Backbone resonance assignments (^{1}H, ^{15}N, ^{13}C) were obtained for more than 90% of all the non-proline residues. Backbone resonance assignments were not obtained for residues R37 (corresponding to R460 of AMA1), D42–D45 (D465–D468), I58 (I481), N76 (N499), K121 (K544), and M122 (M545). A total of 45 rapidly exchanging backbone amide protons were identified from a series of CLEANEX ^{15}N-HSQC spectra (Hwang et al., 1998) recorded on the ^{15}N sample. A total of 35 coupling constants ($^{3}J_{\mathrm{NH}\alpha}$ <6 Hz or $^{3}J_{\mathrm{NH}\alpha} \geq 8$ Hz) was measured from the 3D HNHA experiment.

Deviations of the C^{α} and H^{α} chemical shifts from random coil values and analysis of NOE patterns and $^{3}J_{\mathrm{NH}\alpha}$ coupling constants indicate that domain III is largely unstructured in the N-terminal region and a large portion of the C-terminal region. Structural studies indicate that *Pf* AMA1 domain III forms a structured disulphide-bonded core containing both α-helix and β-sheet secondary structure separated by a largely unstructured loop region. The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4787.

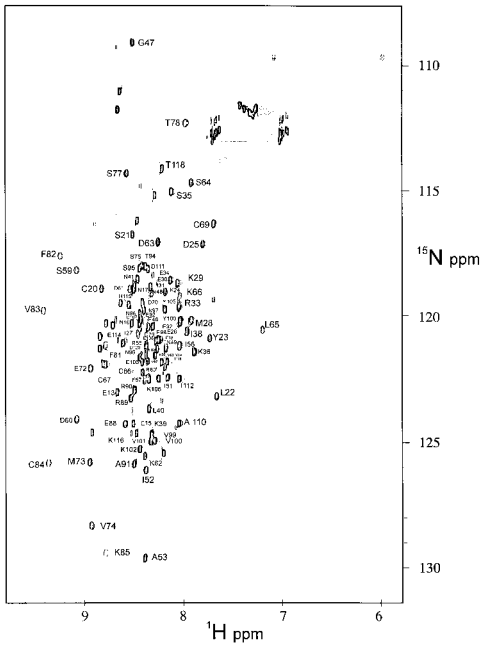

Figure 1. 2D HSQC spectrum of ^{15}N-labelled *Pf* AMA1 domain III in 90% H_2O/10% $^{2}H_2O$ containing 0.05% NaN_3. The sample pH was 3.4 and the spectrum was recorded at 600 MHz and 30 °C. Unlabelled peaks represent either vector-derived residues 1–12 of the 122-residue construct or sample impurities. Solid lines indicate side-chain resonances of Asn residues.

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