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Letter to the Editor: Complete resonance assignments of the C-terminal domain from MIC1: A micronemal protein from *Toxoplasma gondii*

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Biological context

Toxoplasma gondii (T. gondii) is a protozoan parasite that infects up to 90% of adult humans, in whom it causes the life-long chronic infection Toxoplasmosis (Carruthers and Sibley, 1999; Soldati et al., 2001). Microneme discharge is the first step in host invasion and contains both membrane-bound and soluble proteins, which exhibit sequence conservation between genera (Carruthers and Sibley, 1999; Lovett and Sibley, 2003; Tomley and Soldati, 2001). The identification of micronemal proteins from *Plasmodium* merozoites using antibodies that inhibit parasite adhesion to erythrocytes provided the first evidence for the interaction between host cells and micronemal proteins (Miller et al., 1988). It is now generally accepted that the binding of micronemal proteins to host cells provides a molecular bridge to the parasite, thereby facilitating further steps of invasion. The *T. gondii* micronemal protein 1 (TgMIC1) is a soluble adhesive protein found to bind human foreskin fibroblasts and Vero cells *in vitro*. It is a 456 amino acid protein that contain two principle domains; the N-terminal region (TgMIC1-NT) that includes two tandem repeats of ~90 amino acids and the C-terminal region (TgMIC1-CT), which is separated from TgMIC1-NT by a putative hinge region (Fourmaux et al., 1996). Intriguingly, despite suggestion from secondary structure prediction algorithms that a folded domain exists within TgMIC1-CT, it possesses little sequence similarity with any other proteins and therefore cannot be classified.

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Methods

TgMIC1-CT, spanning residues 320 to 456 in TgMIC1, was expressed using the pET 21b plasmid (Novagen) in the BL21 (DE3) *E. coli* strain (Stratagene). ^{15}N, ^{13}C double-labelled samples of TgMIC1-CT were produced in minimal media, containing 0.07% $^{15}NH_4Cl$ and 0.2% ^{13}C-glucose, supplemented with 50 μg/ml carbenicillin. Protein expression was induced by the addition of 50 μM isopropyl-β-D-thiogalactopyranoside.

TgMIC1-CT was purified under denaturing conditions (20 mM Tris–HCL, pH 8.0, 5 mM Imidazole, 0.5 M NaCl and 8 M urea) using the binding of the C-terminal hexahistidine tag to the Ni-NTA HISBind resin (Novagen). Purified protein was refolded by dialysis into 20 mM sodium phosphate buffer, pH 7.0, 150 mM NaCl and concentrated to approximately 0.5 mM for NMR.

The majority of NMR spectra were recorded at 308 K on a 500 MHz four-channel Bruker

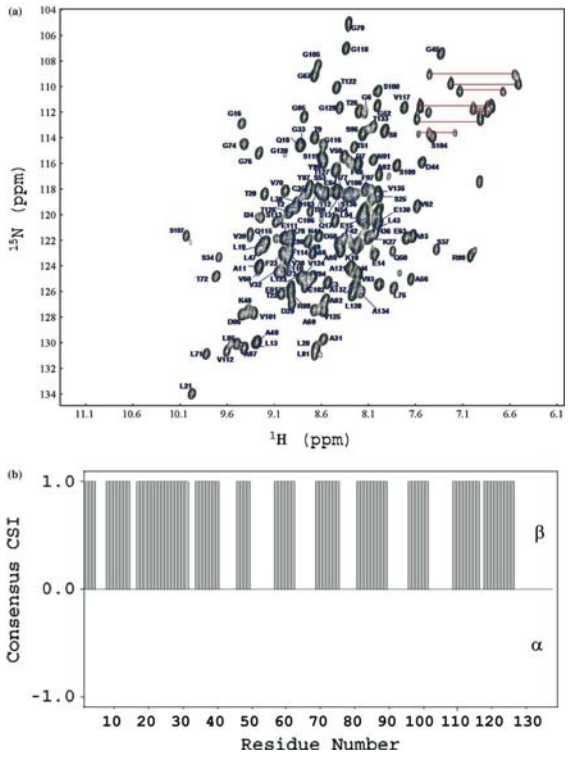

Figure 1. (a) Assigned ^{1}H-^{15}N HSQC NMR spectrum of TgMIC1-CT. Sequential Assignments of the amides are indicated. Asterisks indicate asparagine and glutamine side chain resonances. (b) Chemical Shift Index (CSI) plot (Wishart and Sykes, 1994) of TgMIC1-CT generated using $^{1}H_{\alpha}$, $^{13}C_{\alpha}$, $^{13}C_{\beta}$ and $^{13}C'$ chemical shifts. The β secondary structural elements are indicated. The figure was created using Jplot (c. Jeremy Craven, University of Sheffield).

DRX500 spectrometer equipped with a z-shielded gradient triple resonance cryoprobe. Sequence-specific backbone ^{1}HN, ^{15}N, $^{13}C'$, $^{13}C\alpha$, and $^{13}C\beta$ were determined using standard triple resonance methods (for review see Sattler et al., 1999). H_{α} and H_{β} assignments were obtained using an HBHA(CBCACO)NH experiment (Sattler et al., 1999). All triple resonance experiments employed constant-time evolution in the ^{15}N dimension, whereas in the CBCA(CO)NH and HBHA(CBCACO)NH experiments both indirect dimensions were recorded in constant-time mode. The side chain assignments were achieved using HCCH-total correlation (TOCSY) spectroscopy (Sattler et al., 1999).

Extent of assignments

1D NMR spectra of an initial construct spanning residues 267 to 456 in TgMIC1 revealed a folded domain of approximately 140 amino acids and a highly unstructured region, which was presumed to be the N-terminus. Optimisation of this construct finally generated a product that contains the folded C-terminal domain – TgMIC1-CT. Using the standard triple-resonance assignment methodology, backbone assignments could be made for 98% of the residues from TgMIC1-CT (Figure 1a). The side chain assignments were assessed to be complete with the exception of overlapped regions containing the aromatic, asparagine and glutamine residues. The chemical shift data were used to identify secondary structure elements (Figure 1). These data clearly show the predominance of β-sheet structure in TgMIC1-CT. The C_{β} chemical shifts of the two cysteine residues 35 and 106 indicate that they exist in a fully oxidised form.

A table of backbone and side-chain assignments is available as supplementary material and has been deposited in the BioMagResBank in Madison, WI, USA (accession number 6376).

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