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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C Backbone resonance assignments of the 37 kDa surface antigen protein Bd37 from *Babesia divergens*

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Biological context

The intraerythrocytic protozoa *Babesia divergens* is the agent of the bovine babesiosis in Europe. This Apicomplexa is transmitted to bovine during the blood meal of the tick *Ixodes ricinus*. The parasite inoculated in blood undergoes an asexual life cycle which consists in the intraerythrocytic development of merozoites. The release of newly formed merozoites induces the lyses of the erythrocyte, and causes a haemolytic anaemia, associated with haemoglobinuria and fever. Although the bovine babesiosis is not fatal for most of the cattle, this infection leads to growth and lactation decrease, so that economical losses due to this parasite are sufficient to require the development of a vaccine. Transmission to human is seldom, but can cause a severe and even fatal babesiosis in splenectomised patients (Gorenflot et al., 1998).

In most parasitic protozoa, including *Trypanosoma* and *Leishmania*, the surface of the parasite is coated by glycosylphosphatidylinositol-anchored proteins which are directly involved in host–parasite relationships (Ferguson, 1999). The major merozoite surface antigen of *Babesia divergens*, has been described as a 37 kDa membrane protein (Carcy et al., 1995) GPI-anchored at the surface of the merozoite. The immuno-prophylactic potential of Bd37 has been demonstrated by the protection of animals against babesiosis using injection of an anti-Bd37 monoclonal antibody (Precigout et al., 2004). The determination of the Bd37 structure could significantly improve the understanding of *B. divergens* biology, as well as the rational design of a vaccine. Here we report the backbone assignments of Bd37, as established by triple-resonance experiments.

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Methods and experiments

Protein expression and purification

The cDNA encoding for the 294 residues Bd37 protein from *B. divergenss* was sub-cloned into a *Bam*HI and *Hind*III site of pQE-30 plasmid (Qiagen) allowing expression of the recombinant protein fused to a N-terminal His–Tag. The construct was transformed into *Escherichia coli* M15(pREP4) (Qiagen) and protein expression was induced for 3 h (37 °C) by addition of 1 mM IPTG. The cells were then harvested by centrifugation, and the pellet was sonicated in a lysis buffer (100 mM Tris/HCl, 100 mM NaCl pH 8.5). The supernatant was applied to a Ni-NTA column (Qiagen, Germany). The protein was further purified using ion exchange chromatography using HiTrap Q XL column (Amersham Biosciences) equilibrated with Tris/HCl 20 mM pH 7.5 and eluted with a 0–0.5 M NaCl gradient. Fractions containing Bd37 were

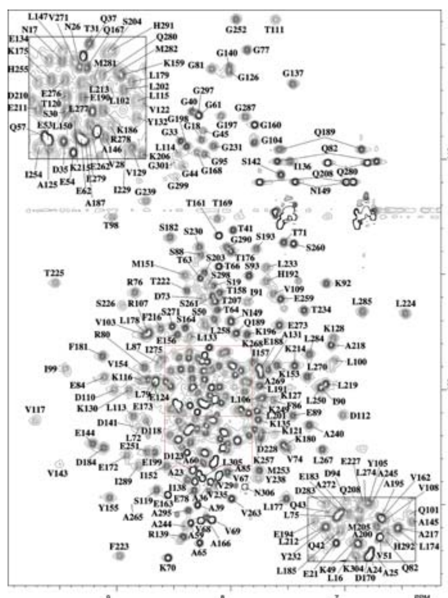

Figure 1. ^{1}H–^{15}N HSQC spectrum of Bd37 from *B. divergenss* recorded at 37 °C on a [^{15}N]-uniformly labelled sample. Cross peak assignments are indicated using the one-letter amino acid and number. The central part of the spectrum is expanded in the two inserts.

pooled and desalted using HiTrap Desalting column (Amersham Biosciences) equilibrated in the final sample buffer (50 mM Sodium Phosphate, pH 6.8, 50 mM NaCl, 10 mM $MgSO_4$) and finally concentrated to 0.8 mM. Uniform ^{15}N/^{13}C and ^{2}H/^{15}N/^{13}C labelling were obtained by growing cells (37 °C) in minimal M9 medium (H_2O or ^2H_2O, respectively) medium containing $^{15}NH_4Cl$ and $^{13}C_6$-glucose as the sole nitrogen and carbon sources, respectively (Eurisotop, St Aubin, France).

NMR spectroscopy

NMR experiments were performed at 37 °C on Bruker AVANCE 600 (HNCO-type experiments) and 800 MHz (HNCA-type experiments) spectrometers equipped with 5 mm *Z*-gradient ^{1}H–^{13}C–^{15}N probes. Backbone and Cβ resonance assignments were made using TROSY-type HNCA, HNCACB, HN(CO)CACB, HNCO and HN(CO)CA experiments (Sattler et al., 1999) performed on the [^{2}H,^{15}N,^{13}C] Bd37 sample. HN and Hα assignments were confirmed using [^{1}H,^{15}N] and [^{1}H,^{13}C] NOESY-HMQC experiments (mixing time 100 ms) recorded on the [^{15}N,^{13}C] Bd37 sample. ^{1}H chemical shifts were directly referenced to the methyl resonance of DSS, while ^{13}C and ^{15}N chemical shifts were referenced indirectly.

Extent of assignments and data deposition

The ^{1}H–^{15}N HSQC spectrum of Bd37 from *B. divergens* is shown in Figure 1. By combining the information from the heteronuclear experiments, we were able to assign more than 98% of the amide group resonances, 91.4% of the other backbone resonances (Cα, C′ and Hα), and more than 95% of the Cβ resonances. Interestingly, the N- and C-terminal segments are not structured, in spite of a disulfide bridge linking their extremities: the corresponding (about 80) residues give rise to the intense cross-peaks centred at 8.3 ppm in the HSQC spectrum. TALOS analysis (Cornilescu et al., 1999) of the (ϕ, ψ) intraresidual dihedral angles, as well as inspection of backbone–backbone nOes, show a predominant content of α-helical structure, yielding a reliable determination of the protein topology. The determination of a low resolution solution structure is in progress, based on the analysis of 'residue-type'-edited NOESY spectra recorded on specifically labeled samples obtained with cell-free technology. Introducing RDC in the structure calculations could further help to refine this low-resolution structure (Wang and Donald, 2004). The chemical shifts have been deposited in the BioMagResBank under the accession number BMRB-6249.

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