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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N NMR backbone assignments of 37 kDa surface antigen OspC from *Borrelia burgdorferi*

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Biological context

Lyme borreliosis is the most common tick-borne infectious disease caused by spirochetes *Borrelia burgdorferi*. The outer surface proteins have been demonstrated to confer full or partial protection against *B. burgdorferi* infection in experimental animals (Probert and LeFebvre, 1994). This had led to development of a vaccine based on outer surface protein A (OspA). However, OspA is preferentially expressed on spirochetes residing within ticks, but it is lost on spirochetes in the mammalian host (Schwan et al., 1995). Thus, OspA-specific immunity is useful for the prevention of Lyme disease, but not for post-infection therapeutic purposes. In contrast to OspA, outer surface protein C (OspC) is not produced by spirochetes in unfed ticks, but its expression is turned on during the blood meal and in the vertebrate host (Schwan et al., 1995). OspC is highly expressed and immunogenic in mammals, and it can elicit protective immunity (Rousselle et al., 1998). We have undertaken an NMR study of the solution structure of OspC. This information, together with epitope mapping data, will guide the development of a new Lyme disease vaccine based on OspC.

Methods and results

A truncated form (residues 19–210) of the OspC gene from *B. burgdorferi* strain B31, in which the leader sequence and the lipidation site have been deleted, was subcloned in pET9a and expressed in *Escherichia coli* BL21 (DE3). Isotope labeling was achieved using standard procedures with ^{15}N-NH$_4$Cl, ^{13}C-glucose and ^{2}H$_2$O. The pelleted cells were suspended in 20 mM Tris-HCl buffer (pH 7.6) containing 100 mM NaCl. After a cycle of freeze and thaw, phenylmethylsulfonyl fluoride (0.5 mM) and hen egg lysozyme (0.5 mg/ml) were added to the suspension, followed by sonication. After centrifugation, the supernatant was passed through a Q-Sepharose FastFlow column (Pharmacia Biotech), and dialyzed against 10 mM sodium phosphate buffer (pH 6.0). OspC was further purified using an SP-Sepharose FastFlow column (Pharmacia Biotech) equilibrated in 10 mM sodium phosphate buffer (pH 6.0) with aN NaCl gradient (0–250 mM). Size-exclusion chromatography and light scattering analyses revealed that OspC exists as a dimer in solution at 50 μM up to 1.5 mM (data not shown). An inspection of the ^{1}H,^{15}N-HSQC spectrum suggested that this construct contains approximately 30 residues that are highly flexible. To identify the location of flexible region(s), we recorded the ^{1}H,^{15}N-HSQC spectrum of a ^{15}N-Lys-labeled protein. The two spectra suggested the presence of an $\sim$21-residue flexible N-terminal tail in OspC. Therefore, we prepared a shorter fragment of OspC corresponding to residues 38 to 210. The structure and stability of OspC(38–210) were similar to those of OspC(19–210) based on circular dichroism (CD) spectra and thermal unfolding profiles monitored by CD (data not shown). The ^{1}H,^{15}N-HSQC spectrum of OspC(38–210) retained all cross peaks belonging to the structural core of the protein. Taken together, these data suggest

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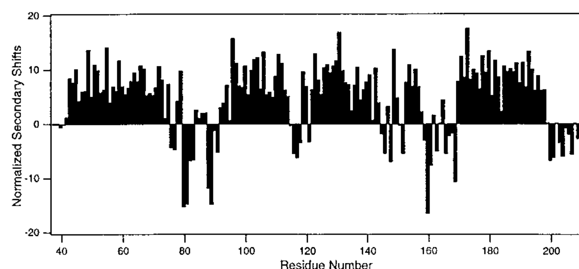

Figure 1. ^{13}C secondary shift analysis of OspC. $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and 13C′ secondary shifts were first normalized to their respective 'significance' values (Wishart and Sykes, 1994), and the normalized values were then summed for each residue. Because the $^{13}C^{\beta}$ shifts exhibit a secondary structure dependent shift in the direction opposite to those of the other nuclei, the $^{13}C^{\beta}$ shifts were negated before summation.

that the N-terminal truncation did not affect the core structure of OspC.

Because of the large size of the protein (∼40 kDa as a dimer), deuteration of non-labile protons was necessary to acquire triple resonance spectra with a sufficient sensitivity. A uniformly ^{13}C, ^{15}N and ^{2}H (≥95%)-labeled NMR sample (1.0 mM) was prepared and dissolved in 20 mM sodium phosphate buffer (pH 6.0) containing 100 mM NaCl, 50 µM EDTA and 1 mM dithiothreitol. All spectra were acquired at 35 °C on a Varian INOVA 600 spectrometer, equipped with a 5 mM triple resonance probe with self-shielded pulsed field gradient. The HNCO, HNCA, HN(CO)CA, HN(CA)CB and HN(COCA)CB spectra were collected with pulse sequences optimized for a highly deuterated sample (Yamazaki et al., 1994a,b; Shan et al., 1996). In addition, the CBCA(CO)CAHA spectrum was collected (Kay, 1993). The acquisition parameters used for each experiment can be obtained from the authors. Spectra were processed using NMR-Pipe software (Delaglio et al., 1995) and analyzed using NMRview software (Johnson and Blevins, 1994) on a Silicon Graphics Indigo2 workstation. Backbone resonance assignments were achieved using standard protocols, mainly relying on the HN(CA)CB and HN(COCA)CB spectra (Shan et al., 1996). ^{13}C secondary shift analysis (Figure 1) identified that OspC contains four helical segments spanning residues 42–78, 93–113, 118–143 and 169–197, while C-terminal residues 198–210 appear to be unstructured.

Extent of assignments and data deposition

Backbone ^{1}HN, ^{15}N, $^{13}C^{\alpha}$ and $^{13}C'$ resonances have been assigned to 98.8% completion, with the only exception being the first two residues and C′ of the third residue. $^{13}C^{\beta}$ assignments are complete except for Ser residues at positions 46, 52, 67, 68 and 194 which were not observable in the HN(CA)CB or HN(COCA)CB spectra. In contrast, $^{13}C^{\beta}$ resonances of the other Ser residues and all Thr residues were unambiguously identified. The missing Ser residues are all located in helical regions (Figure 1). In a helical conformation, the $^{13}C^{\alpha}$ resonance is expected to shift downfield and the $^{13}C^{\beta}$ resonance is expected to shift upfield (Wishart and Sykes, 1994). Because Ser $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ resonances are only 5.5 ppm apart in the random coil configuration, a Ser residue in a helical conformation may well have nearly degenerate $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ resonances. Thus, it is likely that the loss of correlations between the $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ resonances of these Ser residues is caused by inefficient coherence transfer between the C^{α} and C^{β} nuclei due to strong coupling. Chemical shift assignments have been deposited in the BioMagResBank database (accession number 4323).

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