Running Title: **Backbone dynamics and thermodynamics of OspA**

**Backbone Dynamics and Thermodynamics of**

*Borrelia* Outer Surface Protein A

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Summary
Nuclear spin relaxation experiments performed at 298, 308 and 318 K are used to characterize the intramolecular dynamics and thermodynamics of outer surface protein A (OspA), a key protein in the life-cycle of *Borrelia burgdorferi*, the causative agent of Lyme disease. It has recently been demonstrated that OspA specifically binds to the gut of the intermediate tick host (*I. scapularis*), and that this interaction is mediated, at least in part, by residues in the C-terminal domain of OspA that are largely inaccessible to solvent in all X-ray structures of this protein. Our analysis of $^{15}$N relaxation parameters in OspA shows that the putative binding region contains and is surrounded by flexible residues, which could facilitate accessibility to solvent and ligands. In addition, residues with similar activation energies are clustered in a manner that suggests locally collective motions. We have used molecular modeling to show that these collective motions are consistent with a hinge bending mechanism that exposes residues implicated in binding. Characteristic temperatures describing the energy landscape of the OspA backbone are derived from the temperature dependence of the N-H bond vector order parameters, and a comparison is made between the N- and C-terminal globular domains and the unusual single-layer $\beta$-sheet connecting them. The average characteristic temperatures in the three regions indicate that, with an increase in temperature, a larger increase in accessible conformational states occurs for N-H bond vectors in the single-layer central $\beta$-sheet than for bond vectors in the globular N and C-terminal domains. These conformational states are accessible without disruption of hydrogen bonds, providing a conformational entropic gain, upon increase in temperature, without a significant enthalpic penalty. This increase in heat capacity may help to explain the unexpected thermal stability of the unusual single-layer $\beta$-sheet.

Key words: NMR; anisotropy; chemical exchange; dynamics; thermodynamics

Abbreviations: NOE, nuclear Overhauser effect; OspA, Outer surface protein A; NORMAdyn, NMR Optimized Relaxation Modeling with Anisotropy, for dynamics analysis; CPMG, Carr-Purcell-Meiboom-Gill pulse sequence.

Introduction
The Lyme disease associated spirochete, *Borrelia burgdorferi*, expresses a variety of outer surface proteins. Two of these proteins, outer surface proteins A and C (OspA and OspC) are differentially expressed through the life cycle of the spirochete. OspA is up-regulated for transfer of *B. burgdorferi* from infected mice to uninfected ticks (*I. scapularis*), and is down-regulated in favor of OspC for transfer from infected ticks to new uninfected mammalian hosts. Recent studies have shown that OspA mediates spirochete attachment to the tick gut and plays an important role in vector colonization. However, the precise binding target(s) of OspA have not yet been identified. The down-regulation of OspA apparently allows spirochetes to escape the tick gut and disseminate to the salivary glands for transmission to mammalian hosts. Regions of OspA important for binding to tick gut have been identified through peptide competition assays and site-directed mutagenesis, and are found to be associated with a putative ligand-binding site predicted by X-ray crystallography.

The crystal structure of OspA reveals a highly unusual structure (Figure 1). The OspA fold is formed of 21 consecutive anti-parallel $\beta$-strands, and a single C-terminal $\alpha$-helix. The molecule contains a non-globular single-layer $\beta$-sheet connecting an N-terminal $\beta$-sandwich and a C-terminal barrel-like unit (Figure 1A). This central sheet packs against the globular units at either end; however strands 8-11 are largely exposed to solvent on both faces. A putative ligand-binding site in the C-terminal domain was predicted based on structural features and sequence conservation among forty-nine OspA sequences of different *Borrelia* genospecies. The conserved features include a trio of partially buried charged residues and a neighboring solvent-inaccessible cavity lined by hydrophobic residues at the base of a prominent cleft in the C-terminal domain (Figure 1B). These conserved features imply functional importance, such as facilitation or regulation of interactions with binding partners.

The structurally unique central $\beta$-sheet of OspA is surprisingly stable. Hydrogen deuterium exchange rates demonstrate that the conformational stability of this region is comparable to the stability of other globular proteins, and greater than that of the globular C-terminal domain. Measurements of the heteronuclear NOE demonstrate that the central $\beta$-sheet is not highly mobile on the ps time scale. Denaturation studies have demonstrated that this stability is not limited to a single set of conditions. The central sheet is stable over a range...
of temperatures, and unfolds in the same transition as the globular C-terminal domain. Structural features contributing to this stability have been described. Primary features include increased thickness of the central sheet relative to the exposed face of other β-sheets, which allows burial of nonpolar surface area to a degree comparable to small globular proteins, and electrostatic interactions between Glu-Lys cross-strand pairs, which may play an important role in specifying the β-sheet conformation. An important question regarding stability of this region is whether internal motions (i.e., dynamic features) contribute significantly to stability.

NMR experiments have demonstrated that OspA forms a stable monomer in solution at concentrations up to 5 mM, with a global conformation similar to the crystal structure. Contrary to this lack of self-association observed for purified OspA in solution, OspA self-association has recently been observed in ELISA and immunofluorescence assays. These results suggest the presence of a switching mechanism that regulates OspA self-association.

This study of OspA temperature-dependent backbone dynamics is motivated by three key questions: Is there a correlation between the backbone dynamics and the identified functional binding regions? Does the solvent exposed sheet exhibit unusual dynamic properties compared with globular domains? How do the dynamics of OspA affect the stability of the central sheet? We will show that OspA is enriched in μs-ms motions in the region identified as essential for tick gut binding. Based on the temperature dependence of the μs-ms motions, we will propose a conformational switching mechanism that could expose the binding site. We will demonstrate that motions in the solvent exposed β-sheet on the ps-ns and μs-ms time scales are similar to those detected in the globular N and C-terminal domains. We will investigate the temperature dependence of the ps – ns motions in each region, and describe the energy landscape of OspA, noting differences between the globular N and C-terminal domains and the single-layer central sheet. Finally, we will discuss how the dynamic features of OspA may influence its thermal stability.

Results and Discussion

Measurement of 15N relaxation parameters

The backbone dynamics of {15N/2H ("100\%")}-OspA were studied at 298, 308 and 318 K using 15N spin relaxation experiments. The actual level of deuterium incorporated into OspA was approximately 85% based on comparison of the one-dimensional 1H NMR spectrum of this sample vs that of the protonated protein. OspA has 249 backbone amide groups. However, not all peaks could be analyzed at all three temperatures due to spectral overlap or incomplete exchange from deuterium (used in preparation of the protein) to hydrogen. Quantitative analysis was obtained for 210 peaks at 318 K, 189 peaks at 308 K, and 205 peaks at 298 K. The 15N relaxation data (T1, T2 and NOE) are summarized in Table 1, and are also available in full in the Supplementary Material.

Global tumbling of OspA is Anisotropic

The global tumbling parameters calculated for the isotropic and axially symmetric anisotropic models at each temperature are summarized in Table 2. At each temperature, the axially symmetric model is statistically significant. Between temperatures there is good agreement in the size of the anisotropy and the orientation of the unique principal axis. Based on this consistency between temperatures and on the statistical insignificance of full anisotropy for OspA at 318 K, the fully anisotropic model is not further considered here.

Trends in Internal Motions at 318 K

The parameters of internal motion calculated at 318 K are shown in Figure 2. The data for this temperature showed the best resolution and signal to noise ratio. For brevity the 298 and 308 K data are not shown, but are available as Supplementary Material. It is clear from the figure that ps-ns amplitudes of motion are slightly larger (smaller values of the order parameter, S2) in the first five β-stands of the N-terminal region (residues 24 – 80), and are relatively constant across the remainder of the protein. The density (in sequence space) of residues influenced by motions on the ns time scale (τint > 0.5 ns) decreases from the N to the C-terminus, and the density of residues requiring inclusion of a chemical exchange term, Rex, increases from the N to the C-terminus. Inclusion of a chemical exchange term indicates time-dependent changes in the chemical environment surrounding a nucleus that occur on the μs-ms time scale. The observed trend in the density of chemical exchange terms is consistent with the trend in the values of the free energy of opening of a hydrogen-bonded amide proton, ΔG_HDX, observed in hydrogen deuterium exchange measurements by Pham et al.,. Values of ΔG_HDX decrease from the N to the C-terminus, with lower values of ΔG_HDX indicating regions of increased conformational flexibility that facilitate exchange with solvent.

The Backbone is Flexible Around the Putative Binding Site

Several proteins have been found to display enrichment of μs-ms time scale backbone motions in binding surfaces, suggesting an important role for such motions in molecular recognition. Recent experiments have shown that OspA mediates spirochete attachment to the gut of its tick host, and have identified a functional binding site in residues 229-247. A peptide
containing residues 229-247 competes with full-length OspA in binding to tick gut. Further, OspA mutants with replacements in amino acids 236-7 or 242-4 demonstrated significantly less binding to tick gut than wild-type. Phe-237 and Ile-243 are associated with the buried hydrophobic cavity that, along with three neighboring partially buried charged residues, was previously suggested to participate in ligand binding. The chemical exchange parameters obtained for OspA at 318 K are shown mapped onto the crystal structure in Figure 3. It is evident that this putative binding region is surrounded by residues affected by motions on the μs-ms time scale. The buried nature of the hydrophobic cavity in the crystal structure suggests that this flexibility is necessary in vivo to make the binding site accessible to a ligand.

Temperature Dependence of Motions on the μs-ms time scale

The temperature dependence of the exchange terms was used to define lower bounds for the microscopic exchange rate constants (kex), and to estimate the energy barriers associated with a (presumed) two-state exchange model. As noted by Millet et al., the accuracy of this estimation is limited: temperature changes perturb the populations of the two states, and can affect Δω through temperature-dependent conformational changes. However, numerical calculations, performed by Mandel et al., indicate that Eω is underestimated by less than a factor of two.

For OspA, with the exception of Glu-77, the chemical exchange terms decrease with increasing temperature, indicating that the microscopic exchange rate, kex, is faster than 3.2/τCP, or kex > 3.6 × 10^7 s^-1 (τCP = 0.9 ms). Arrhenius plots of ln(Rex) versus 1/T were used to determine apparent activation energies for those residues that required an exchange term at all three temperatures. These activation energies are presented in Figure 4 in relationship to the secondary structure. Residues with values of Rex < 0.75 s^-1 at 318 K are not shown, since the contribution from chemical exchange is of the size of the average error in T2, and may not be reliably determined. Spatially clustered residues in strands 12 – 19 display similar activation energies, suggesting a collective conformational transition. The mean activation energy in this region is 44 kJ/mol, with a standard deviation of 16 kJ/mol. Collective motions on sub-millisecond time scales in proteins have been inferred previously. Residues with significantly lower activation energies include Gly-183, Val-199, and the loop between strands 20 and 21. These residues appear to undergo additional local transitions with significant chemical exchange, which dominate the activation energies. For example, the observed chemical exchange values for Val-199 are likely to be dominated by wobble of the Trp-216 side chain, to which the Val-199 NH is hydrogen bonded.

The clustering of activation energies on either side of strands 16 – 17 can be rationalized by a mechanism of motion supported by structural features in this region. Besides the connecting hydrogen bonded turn, β-strands 16 and 17 interact via a single, unusual hydrogen bond between the backbone NH of Val 199 and the aromatic ring of Trp-216 (V199NH ··· W216ring). These two strands are located on opposite sides of an interface between a sub-domain (containing β-strands 17 - 21 and the C-terminal α-helix) and the rest of OspA. This interface also contains the hydrophobic cavity, the three partially buried charged residues, and residues identified via mutational analysis as being critical for tick gut binding.

The observed dynamics and activation energies are consistent with a hinge-opening motion in which the sub-domain separates from the interface, as depicted in Figure 5(a and b). A crankcase motion of the Lys-212, Thr-213 peptide plane coupled with rotation about ψ of Thr-213 displaces the sub-domain and exposes the interface, while adjustment of Trp-216 χ1 and χ2 maintains the V199NH ··· W216ring hydrogen bond. This is consistent with the high protection factor associated with the amide proton of Val-199 (ΔG = 25 kJ/mol, Pham et al. NSB 1998). The crux of the hinge at Lys-212 and Thr-213 is supported by the observation of chemical exchange in the beginning of strand 17, while rotation of the Trp-216 side chain is supported by the observation of chemical exchange in the facing residues of strand 16 (Figure 3). The broad spatial distribution of activation energies throughout the sub-domain and strands 12 – 16 suggests that exposure of the interface induces conformational readjustment and changes in chemical environment throughout this region. Most of the secondary structural elements in these regions contribute residues directly to the interface. Hence, this structure-based model is fully consistent with the available dynamic data.

The tick gut binding function of OspA requires interaction with a region that is buried in the crystal structure. This closed form is the dominant structure in solution, based on chemical shift values, homonuclear NOEs (Shohei Koide, unpublished results), hydrogen deuterium exchange rates and SAXS (Bu et al. 1998). Interaction between OspA and its target is likely to require exposure of this buried region. Hence, the proposed motion is fully consistent with the functional requirements, since it provides a mechanism for significant exposure of the putative binding region.

A mechanism for further stabilization of the open state upon ligand binding is suggested by the crystal structure. Examination of the 1osp structure shows that the gap between β-strands 16 and 17 can be reduced through small rotations of the backbone torsion angles in the β-
The Backbone Displays Similar Motions in the Solvent Exposed \( \beta \)-Sheet and the Globular N and C-terminal Domains

Previous experiments have demonstrated that the central \( \beta \)-sheet is not highly mobile on the ps time scale, and is stable on the s – hr (HDX) time scale \(^{9,10} \). While it might be expected that a \( \beta \)-sheet exposed to solvent on both faces would be enriched in motions on a range of time scales, similar to the motions often observed in hydrogen bonded \( \beta \)-turns, the data shown in Figure 2 demonstrate that this is not the case. The amplitude of ps motions are similar across the majority of the protein, the density of ns motions in the central sheet (residues 111 – 147) is lower than the density of ns motions in the globular N terminal region, and the density of \( \mu \)-ms motions is lower in the central sheet than in the globular C terminal region. These results indicate that the single-layer \( \beta \)-sheet architecture can reduce the rapid motion of the peptide backbone to a degree similar to that achieved in a globular protein.

Temperature dependence of motions on the ps – ns time scale

Thermodynamic parameters associated with backbone fluctuations on the ps – ns time scale were estimated from the temperature dependence of the order parameters, as described in Materials and Methods. OspA is well-ordered on the ps – ns time scale, as demonstrated in Figure 2 for 318 K and in Supplementary Material for 308 and 298 K. As a result, calculations of the characteristic temperature, \( T^* \), defined for small deflections in an axially symmetric parabolic potential, are more appropriate than calculations of \( C_p \), which can be susceptible to errors for values of \( S^2 \) > 0.8 \(^{27,28} \). Initial calculations of \( T^* \) and \( C_p \) produced fits at negative temperatures and heat capacities for some residues, in violation of the physical model. For these residues, the expected decrease in \( S^2 \) with increasing temperature \(^{29} \) was not observed. Rather, \( S^2 \) increased with increasing temperature for those residues influenced by chemical exchange. While it is possible that the motion of a particular residue might become more restricted upon an increase in temperature, a survey of the literature suggests another explanation of this phenomenon. This behavior has been observed by others and attributed to a negative correlation between fitted values of \( S^2 \) and \( R_{ex} \) \(^{20,21,29,30} \).

To correct for the artifactual reduction in \( S^2_{\text{fit}} \), the correction factor proposed by Mandel et al., \(^{20} \) was applied, as described in Materials and Methods. The weighted mean order parameters and \( T^* \) values for each \( \beta \)-sheet and the C-terminal helix are reported in Supplementary Information for uncorrected and corrected values of \( S^2 \). We find that this correction results in values of \( S^2 \) that decrease with increasing temperature, and the corrected values of \( S^2 \) are used in the calculation of all thermodynamic parameters reported herein. Weighted mean order parameters for residues in secondary structures in the N-terminal (strands 1-7, globular), central (strands 8-11, solvent exposed) and C-terminal (strands 12-21 and helix, globular) regions of OspA are presented in Table 3. Characteristic temperatures are presented in Table 4 along with comparative data for three other proteins for which characteristic temperatures have been reported.

The characteristic temperature, \( T^* \), parameterizes the distribution of thermally accessible conformational states and is closely connected to the heat capacity of a conformational mode. When \( T^*/T \gg 1 \), few additional conformational states are populated upon an increase in temperature; the mode has low heat capacity because few states are thermally accessible \(^{20} \). The characteristic temperature of secondary structural elements in all three regions of OspA is noticeably smaller than in ribonuclease H (RNaseH) and the troponin C (TnC) regulatory domain, proteins for which the conformational dynamics of the backbone contribute little to the heat capacity of the folded state \(^{20,31} \). In contrast, the OspA \( T^* \) values are much more similar to those obtained for the B1 domain. For the B1 domain, backbone motions have been found to contribute significantly to the heat capacity of the folded state \(^{32} \).

The characteristic temperature of the central region of OspA is significantly smaller than the characteristic temperatures of the N and C-terminal regions. (The probability of obtaining a measurement of 830 K given a true value of 675 K ± 70 K is less than 5%. The probability of obtaining a measurement of 675 K given a true value of 830 K ± 43 K is less than 0.5%.) Calculations of \( C_p \) for each region (data not shown) display the expected trends based on \( T^* \). The low \( T^* \) values indicate that the contribution of the backbone dynamics to the heat capacity is large for all regions of OspA, and is significantly larger in the central region than in the N- and C-terminal regions. We note that this
increase in heat capacity does not necessitate a large decrease in enthalpy, since the amplitudes of ps-ns motion at 318 K would not require breakage of hydrogen bonds. For example, a change in $<S^2>$ from 0.98 to 0.90 corresponds to an increase in cone solid angle $<\theta>$ from 7° to 15° for the diffusion in a cone model. Using a recently reported hydrogen bond potential, deviations of 7° and 15° away from an optimal hydrogen bond angle retains 97% and 87% of the optimal hydrogen bond energy, respectively.

The stability of the folded state of a protein is determined by

$$\Delta G_{\text{unfold}} = \Delta H_0 - T \Delta S_0 + \Delta C_{p,\text{unfold}} \left( T - T_0 - T \ln \left( T/T_0 \right) \right)$$

in which $\Delta H_0$ and $\Delta S_0$ are the enthalpy and entropy changes, respectively, in going from the folded to the unfolded state at a reference temperature $T_0$, and $\Delta C_{p,\text{unfold}}$ is the change in heat capacity at constant pressure, which is assumed to be invariant with temperature. A folded state may be stabilized by an increase in $\Delta H_0$ or by a decrease in either $\Delta S_0$ or $\Delta C_{p,\text{unfold}}$. The enthalpic contributions to the stability of the central sheet of OspA have been described elsewhere. The current analysis indicates that the greater heat capacity of the central sheet, manifested as a greater temperature sensitivity of backbone motions, may contribute to its thermal stability by decreasing $\Delta C_{p,\text{unfold}}$.

Concluding Remarks

The studies reported herein provide a clear example of how NMR relaxation studies can reveal important relationships between structure, dynamics and function. $^{15}$N spin relaxation experiments performed at 298, 308 and 318 K have been used to characterize the intramolecular dynamics and thermodynamics of OspA. An elegant mechanism by which OspA may change conformation to carry out its role in attachment to tick gut is revealed by the temperature dependence of $\mu$s-$\mu$s time scale motions. The temperature dependence of ps–ns time scale motions reveals an important entropic contribution to the stabilization of the unique single-layer $\beta$-sheet.

Recent experiments have demonstrated that OspA specifically binds to the gut of *I. scapularis*, and that this binding is mediated, at least in part, by a putative binding region in the C-terminus of the protein. Residues known to be important for binding are inaccessible to solvent in the crystal structures of OspA (1osp, 1fj1) and lie in or near an interface between a C-terminal sub-domain and the main body of the protein. We find that this interface is surrounded by residues affected by motions on the $\mu$s-$\mu$s time scale, and that clustering of activation energies around the interface suggests a locally collective motion.

We find structural and dynamic evidence to be consistent with motion of a sub-domain composed of strands 17 – 21 and the C-terminal helix. Such a motion would expose the putative binding region of OspA, facilitating interaction with its binding partner(s). This binding region represents a potentially important target for prevention of Lyme disease, since inhibition of OspA binding to tick gut would interrupt an important step in progression of *B. burgdorferi* through its intermediate host.

The temperature dependence of the backbone order parameters has been parameterized by a characteristic temperature that describes the energy scale associated with the distribution of bond vector conformations. We find that the order parameters are strongly correlated with chemical exchange parameters, and that a correction term, proposed by Mandel et al., 1996, must be applied to obtain a physically reasonable correlation between order parameter and temperature. A significantly lower characteristic temperature for the solvent exposed $\beta$-sheet indicates that more conformational states are accessible to N-H bond vectors in the secondary structural elements of this sheet than are accessible to those in the globular N and C-terminal domains. These conformational states are accessible without disruption of hydrogen bonds. The correspondingly higher heat capacity may help to thermally stabilize the unusual structure.

While it has been shown that backbone conformational entropy can contribute to protein stability, the role of side chain dynamics in modulating protein stability may be significant, and is not yet fully understood. Analysis of the contributions of OspA side chains to the heat capacity of the folded state may, in the future, significantly improve our understanding of the role of dynamics in determination of the stability of the solvent exposed $\beta$-sheet. In addition, identification and purification of the ligand binding partner of OspA would allow an analysis of the role of dynamics in the interaction, providing additional insight into the function of OspA invector colonisation.

Materials and Methods

**Preparation of OspA NMR sample**

$^2$H,$^{15}$N-labeled OspA was prepared as described previously. The NMR sample was composed of a 1.5 mM solution of labeled OspA in sodium phosphate buffer (10mM, pH 6.0 at room temperature) containing sodium chloride (50mM), EDTA (50 mM) and sodium azide (0.02%) in 95% H$_2$O/5% D$_2$O.

**NMR Experiments and Data Processing**

NMR experiments on OspA were performed at 298, 308 and 318 K on a Varian Unity INOVA 600 spectrometer using a 5 mm self-shielded triple resonance...
probe with a z-axis pulsed field gradient. The temperature in the sample region of the probe was calibrated using a 100% methanol sample and verified using a 100% ethylene glycol sample.

Backbone amide $^1$H-$^1$H NOE, and $^{15}$N T$_1$ and T$_2$ (CPMG) values were measured for OspA using published procedures. At all temperatures the T$_1$ relaxation decay was sampled at 7 different time points (.0110, .0220, .0550, .110, .220, .550, and 1.10 s), with one duplicate recorded at .011 and .110 s for 308 and 318 K, and two duplicates recorded at these points for 298 K. At 308 and 318 K the T$_2$ decay was sampled at 4 different time points (.0165, .0329, .0658, and .115 s), with one duplicate recorded at .0165 s and two duplicates recorded at .115 s. At 298 K the T$_2$ decay was sampled at 5 different time points (.0165, .0329, .0658, .0987 and .115 s), at least one duplicate was recorded at each time point, with two duplicates recorded at .0165 s and .115 s. The interpulse delay between $^{15}$N 180° pulses in the CPMG sequence was 0.9 ms. All experiments were performed using spectral widths of 2.13 x 9.00 kHz in the t$_1$ x t$_2$ dimensions. T$_1$ and T$_2$ measurements used a recycle delay of 1 s; NOE measurements used a recycle delay of 9.4 s, and a $^1$H saturation length of 4.6 s. T$_1$, T$_2$ and NOE measurements were performed using a total of 32 transients per t$_1$ experiment. For T$_1$, T$_2$ and NOE, 512 x 1024 complex points were acquired in the t$_1$ x t$_2$ dimensions. Resonance assignments for OspA at 318 K have been previously reported. Assignments of backbone $^1$H and $^{15}$N resonances at 308 and 298 K were determined by monitoring peak shifts in the $^{15}$N-$^1$H HSQC spectrum as a function of temperature, with spectra recorded at 298, 301, 304, 308, 311, 314 and 318 K. All NMR data were processed and analyzed using the NMRPipe and nmrDraw software tools by propagation of errors. Uncertainties in peak height measurements were estimated from noise in the baseplane, and uncertainties in NOE values were obtained by propagation of errors.

**Analysis of Parameters of Motion**

For accurate determination of global tumbling parameters, it is necessary to remove from the analysis peaks with significant contributions to relaxation from ns or µs-ms time scale internal motions. For data at 318 K and 308 K, a two step filtering process optimized for proteins with large anisotropy was applied. For data at 298 K, lower signal to noise and increased peak overlap complicated the filtering process. The data at 298 K was therefore filtered in a single step by comparison to data at 308 K. All residues that did not fit Model 2 (vide infra) with $\tau_c < 600$ ps at 308 K were removed from the data set at 298 K, and subsequent analysis was performed as described elsewhere.

After determination of the global tumbling parameters, the parameters of internal motion were calculated using the spectral density function for anisotropic rotational diffusion. The calculated values were obtained by using the standard five models for internal motion described in and used in ModelFree, although the program ModelFree was not used for the analysis (i.e., Model 1 fits S$^2$ only; Model 2 fits S$^2$, $\tau_c$; Model 3 fits S$^2$, $\tau_c$, $\tau_f$; Model 4 fits S$^2$, $\tau_c$, $\tau_f$; Model 5 fits S$^2$, S$^2$, $\tau_f$). Two types of model selection were performed. For analysis of data at a single temperature, a traditional model selection protocol was followed. The one-parameter model was chosen when $\chi^2 < 9.2$ (0.01 critical value for two degrees of freedom) and a two-parameter model did not provide a significantly better fit (F < 9.5, 0.2 critical value). The two-parameter model was chosen when $\chi^2 < 6.6$ (0.01 critical value for one degree of freedom), and when it provided a significantly better fit (F > 9.5) than the one-parameter model. Three parameter models were invoked only if the one- and two-parameter models did not provide acceptable fits, and if the three-parameter model could be fit with $\chi^2 = 0$. For analysis of data across multiple temperatures, the model selection protocol was altered to determine the best fit model at all three temperatures. Any “fit” model ($\chi^2 < 9.2$, $\chi^2 < 6.6$, and $\chi^2 = 0$ for 2, 1, and 0 degrees of freedom, respectively) was considered. The model with the smallest number of fitted parameters that provided agreement at the maximum number of temperatures was selected for each residue.
All of the analysis, starting from values of $T_1$, $T_2$ and NOE and ending with internal motion parameters, was performed using a suite of programs collectively referred to as NORMAdyn (NMR; Optimized Relaxation Modeling with Anisotropy, for dynamics analysis) (N.H. Pawley and L.K. Nicholson, Cornell University).

Reliability of Chemical Exchange Parameters

We have assessed the reliability of chemical exchange parameters in multiple ways. First, to ensure that putative motions on the $\mu$s – ms time scale are not an artifact of differing domain orientations in the crystal and solution structures, the parameters of global tumbling and internal motion were optimized for the C-terminal domain alone 49. Optimizing the C-terminal region alone did not significantly affect the parameters of global tumbling or chemical exchange, as described in detail elsewhere 53. Second, chemical exchange terms are found to be consistent between separate analyses for data sets from two different OspA samples (1H,15N-labeled OspA and 1H,14N-labeled OspA, data not shown). Third, assessment by the group of Garcia de la Torre using HydroNMR 50,51 demonstrates the same trend of increased chemical exchange parameters from the N to the C-terminus (through values of $T_1/T_2$ that were higher in the experimental data than the calculated data 52). Fourth, correlation between values of $R_{ex}$ and N-H bond vector orientation were found not to be statistically significant (correlation coefficient = 0.23). Finally, chemical exchange parameters are found to be consistent between the three temperatures, and the magnitude of the chemical exchange terms increases with decreasing temperature, as expected (vide infra). Based on these assessments, we conclude that the existence, location and magnitude of these $\mu$s-ms motions are reasonable and reproducible within experimental error.

Analysis of Thermodynamic Parameters

Thermodynamics parameters were obtained from the parameters of internal motion using established relationships. A characteristic temperature, $T^*$, describes the density of energy states thermally accessible to a particular bond vector, and is defined by 20:

$$\frac{1}{T^*} = \left(\frac{2}{3}\right)\frac{d(1-S)}{dT}$$

where $S$ is the order parameter, and $T$ is the absolute temperature.

The apparent activation energy for a given exchange process, $E_a$, is defined by 20,21:

$$E_a = \frac{d\ln(R_{ex})}{d(1/RT)}$$

where $R_{ex}$ is the rate of transverse relaxation due to the exchange process, $R$ is the gas constant, and $T$ is the absolute temperature.

The entropy, $S_B$, associated with motions on the ps – ns time scale is approximated by 27:

$$S_B = k_B \ln\left\{\pi\left(3 - \sqrt{1 + 8S}\right)\right\}$$

where $k_B$ is Boltzmann’s constant, and $S$ is the order parameter. Finally, the heat capacity at constant pressure, $C_p$, is defined by 27,28:

$$C_p = \frac{\partial S_B(T)}{\partial \ln T}$$

where $C_p$ is the heat capacity at constant pressure, and $S_B(T)$ is the entropy at temperature $T$. Values of $C_p$ are discussed qualitatively rather than presented quantitatively since $C_p$ estimates will be susceptible to large errors for OspA due to the breakdown of the classical model for large values of $S^2$ and the rapid changes in entropy with respect to order parameter for $S^2 > 0.8$ 27,28.

Correlation between $S^2$ and $R_{ex}$

A negative correlation between the fitted values of $S^2$ and $R_{ex}$ has been observed (vide supra) and noted by others 20,21,29,30. A correlation between $S^2$ and $R_{ex}$ would result in correlation between the changes in these parameters with temperature, and potentially artificial values of the extracted thermodynamic parameters. To investigate the negative correlation between $S^2$ and $R_{ex}$ as an explanation for a positive correlation between $S^2$ and $T$ (vide infra), synthetic data sets were constructed and analyzed as described elsewhere 13, with the exception that $R_{ex}$ values were drawn from the distribution function

$$P(R_{ex}) = e^{-(R_{ex}-R_{ex0})^2/2\sigma_{Rex}^2} \cdot H(R_{ex})/\Gamma,$$

where $R_{ex0} = 3$ s$^{-1}$, $\sigma_{Rex} = 1.5$ s$^{-1}$, $H(x)$ is the Heaviside step function, and $\Gamma$ is a normalization constant.

Values of $T_1$, $T_2$ and NOE were calculated from the synthetic parameters of motion, and analyzed by the same process as applied to experimental data. As would be expected, in the absence of error in the relaxation parameters, the fitted values of $S^2$ and $R_{ex}$ are the same as the true values, and there is no correlation between these fitted parameters. The effects of experimental error were simulated by adjusting the values of $T_1$, $T_2$ and NOE with the addition of the gaussian-distributed random variables $\delta_{T1}$, $\delta_{T2}$ and $\delta_{NOE}$. These random variables were drawn from a gaussian distribution of width 2 % $\times$ $T_1$ for $\delta_{T1}$ and 3 % $\times$ NOE for $\delta_{NOE}$, to reflect the expected percent error in experimental data (see Table 1). In the presence of noise, the fitted values of $S^2$ and $R_{ex}$ are no longer the
same as the true values, and \( S_{2_{\text{fit}}} - S_{2_{\text{true}}} \) is found to be very highly (negatively) correlated with \( R_{\text{exSS}} - R_{\text{extrue}} \) (\( P = 6 \times 10^{-21}, 3 \times 10^{-17} \) and \( 2 \times 10^{-18} \) for three separate synthetic data sets). While the best correlation is obtained when error-free “true” parameters are known, the correlation between \( S_{2_{\text{fit}}} - S_{2_{\text{true}}} \) and \( R_{\text{exSS}} \) for the same data sets is also significant, exceeding the 95% confidence limit for each of the three synthetic data sets (\( P = 1.4 \times 10^{-2}, 4.5 \times 10^{-3} \) and \( 2.5 \times 10^{-5} \)). This implies that, in the absence of knowledge of the error-free “true” parameters, the correlation between \( S_{2_{\text{fit}}} \) and \( R_{\text{exSS}} \) can be used to improve the estimate of \( S_{2_{\text{true}}} \).

To correct for the artifactual reduction in \( S^2 \) and improve our estimate of \( S_{2_{\text{true}}} \) we applied the correction factor proposed by Mandel et al. \(^{20} \), of

\[
S_c^2 = S_0^2 + 0.018 R_{\text{ex}},
\]

with an associated increase in the error in \( S^2 \) given by

\[
\sigma_{S_c^2} = \sigma_{S_0^2} + 0.004 R_{\text{ex}},
\]

where \( S_0^2 \) is the corrected value of \( S^2 \), \( S_0^2 \) is the original value of \( S^2 \), and \( \sigma_{S_0^2} \) represents the error in \( S_0^2 \). The corrected values of \( S^2 \) were used in the calculation of all reported thermodynamic parameters.

Acknowledgments

We thank Shude Yan for preparing the \(^{15}\text{N}, ^{2}\text{H}\)-labeled OspA sample, Jason D. Gans for valuable comments and discussion, Lewis E. Kay for use of his pulse sequence library, and Frank Delaglio and Dan Garrett (NIH/NIDDK) for use of their NMR software tools. This research was funded by the NSF MCB-9808727 (L.K.N.), and NIH R01-GM57215 (S.K.). Fellowship support for N.H.P. was provided by the National Physical Science Consortium and the Department of Energy through Lawrence Livermore National Laboratory.

References


Table 1. Average OspA$^{15}$N spin relaxation rate constants and errors

<table>
<thead>
<tr>
<th></th>
<th>298 K</th>
<th>308 K</th>
<th>318 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average $T_1$ (s)</td>
<td>1.38 ± 0.037\textsuperscript{a}</td>
<td>1.11 ± 0.050</td>
<td>0.961 ± 0.035</td>
</tr>
<tr>
<td>Average $T_2$ (s)</td>
<td>0.0393 ± 0.00076\textsuperscript{a}</td>
<td>0.0496 ± 0.0011</td>
<td>0.0619 ± 0.0013</td>
</tr>
<tr>
<td>Average NOE</td>
<td>0.693 ± 0.012</td>
<td>0.703 ± 0.012</td>
<td>0.716 ± 0.015</td>
</tr>
</tbody>
</table>

\textsuperscript{a} In addition to the Monte Carlo error analysis described in Materials and Methods, repeat data sets were measured for $T_1$ and $T_2$ at 298 K. For 171 resolved peaks, the average rmsd between repeat $T_1$ data sets was 1%, and the average rmsd between $T_2$ repeat data sets was 2%.
Table 2. Global tumbling parameters for OspA at three different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Model</th>
<th>( \tau_{c,\text{eff}}^a ) (ns)</th>
<th>( D_{\perp} ) (ns(^{-1}))</th>
<th>( D_{\parallel} ) (ns(^{-1}))</th>
<th>( D_{\parallel} / D_{\perp} )</th>
<th>( \theta ) (°)  (^b)</th>
<th>( \phi ) (°)  (^b)</th>
<th>( \chi^2 ) / ( \nu )  (^c)</th>
<th>F</th>
<th>( p^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298 K</td>
<td>isotropic</td>
<td>17.3</td>
<td>0.00965</td>
<td>0.00965</td>
<td>------</td>
<td>------</td>
<td>70.1</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>axially</td>
<td>17.5</td>
<td>0.00694</td>
<td>0.0146</td>
<td>2.2</td>
<td>177</td>
<td>160</td>
<td>12.4</td>
<td>98.9</td>
<td>3.45E-23</td>
</tr>
<tr>
<td>308 K</td>
<td>isotropic</td>
<td>14.7</td>
<td>0.0113</td>
<td>0.0113</td>
<td>------</td>
<td>------</td>
<td>53.6</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>axially</td>
<td>14.6</td>
<td>0.00801</td>
<td>0.0182</td>
<td>2.3</td>
<td>177</td>
<td>145</td>
<td>12.5</td>
<td>90.3</td>
<td>2.69E-25</td>
</tr>
<tr>
<td>318 K</td>
<td>isotropic</td>
<td>12.4</td>
<td>0.0135</td>
<td>0.0135</td>
<td>------</td>
<td>------</td>
<td>72.4</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>axially</td>
<td>11.9</td>
<td>0.00996</td>
<td>0.0222</td>
<td>2.1</td>
<td>178</td>
<td>121</td>
<td>11.2</td>
<td>195</td>
<td>1.50E-42</td>
</tr>
</tbody>
</table>

\(^a\) \( \tau_{c,\text{eff}} \) is calculated as \((1/2)(2D_{\perp} + D_{\parallel})\)^\(^{-1}\), where \( D_{xx} = D_{yy} = D_{\perp} \) and \( D_{zz} = D_{\parallel} \). The \( D_{ii} \)'s are the magnitudes of the principal components of the diffusion tensor.

\(^b\) The angles \( \theta \), \( \phi \), and \( \psi \) define the orientation of the diffusion tensor with respect to the moment of inertia frame of the X-ray crystallographic structure of OspA. For axially symmetric diffusion, \( \psi \) is defined to be zero. Note that for small \( \theta \), \( \chi^2 \) is only weakly dependent on \( \phi \).

\(^c\) Results were calculated using 64, 82, and 108 residues at 298, 308, and 318 K, respectively. Values of \( \nu \) (number of degrees of freedom, \textit{i.e.}, number of data points – number of fitted parameters) are therefore 63 and 60, 81 and 78, 107 and 104 for the isotropic and axially symmetric tumbling models at 298, 308, and 318 K, respectively.

\(^d\) Fully anisotropic tumbling was shown elsewhere \(^1\) not to be statistically significant for OspA at 318 K.
Table 3. Weighted Mean Order Parameters of OspA by Region

<table>
<thead>
<tr>
<th>Region</th>
<th>Secondary Structures</th>
<th>$\langle S^2 \rangle^a$ 318 K</th>
<th>$\langle S^2 \rangle^a$ 308 K</th>
<th>$\langle S^2 \rangle^a$ 298 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>N terminal</td>
<td>Strands 1-7</td>
<td>0.86 ± 0.003 (27)</td>
<td>0.86 ± 0.003 (24)</td>
<td>0.92 ± 0.002 (28)</td>
</tr>
<tr>
<td>Central</td>
<td>Strands 8-11</td>
<td>0.90 ± 0.004 (12)</td>
<td>0.95 ± 0.005 (9)</td>
<td>0.98 ± 0.008 (8)</td>
</tr>
<tr>
<td>C terminal</td>
<td>Strands 9-21 &amp; Helix</td>
<td>0.89 ± 0.002 (48)</td>
<td>0.92 ± 0.003 (45)</td>
<td>0.96 ± 0.003 (45)</td>
</tr>
</tbody>
</table>

*Weighted mean order parameters and uncertainty in the mean, calculated from the corrected values in Table S2 of the Supplementary Material. The number of residues averaged for each entry is given in parentheses. Only those residues that fit the same time scale of motion at all three temperatures (for this purpose Models 3 and 4 are treated as the same model) were used in the calculation.*
<table>
<thead>
<tr>
<th>Protein</th>
<th>Average over</th>
<th>T* (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OspA</td>
<td>N terminal region</td>
<td>830 ± 43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Central region</td>
<td>675 ± 70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>C terminal region</td>
<td>844 ± 46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B1 domain&lt;sup&gt;32&lt;/sup&gt;</td>
<td>All Beta Sheets</td>
<td>573 ± 265&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Single Alpha Helix</td>
<td>652 ± 160&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RNase H&lt;sup&gt;20&lt;/sup&gt;</td>
<td>All Secondary Structures</td>
<td>2500 ± 300&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3700 ± 540&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Troponin C Regulatory domain&lt;sup&gt;31&lt;/sup&gt;</td>
<td>All Secondary Structures</td>
<td>2200 ± 95&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> T* values are calculated from the weighted mean order parameters of Table 2. Uncertainties associated with T* values were estimated by Monte Carlo simulation.

<sup>b</sup> Average and standard deviation of T*.

<sup>c</sup> T* = 3700 K when all residues are included in the fit. When residues fit with chemical exchange terms are excluded, T* = 2500 K. Values of T* were calculated from reported values of d(1-S)/dT.

<sup>d</sup> Values of T* were calculated from reported values of d(1-S)/dT.
Figure 1. Crystal structure of OspA from 1osp. a) Ribbon diagram of OspA showing single-layer central sheet. Ribbon prepared with WebLab Viewer (Accelrys Inc). b) OspA surface showing putative ligand binding site. The partially buried charged residues are shown in blue (Arg-139, Lys-189) and red (Glu-160), residues forming the hydrophobic cavity are shown in green (Leu-167, Leu-178, Leu-187, Phe-237, Ile-243). Surface generated using Qmol (Gans and Shalloway, 2001).
Figure 2. Dynamics parameters plotted vs. residue number for OspA at 318 K. Secondary structural elements (21 β-strands and C-terminal helix) are shown as gray bars. a) Values of $S_2^2$ are equivalent to $S_f^2$ for Model 2 and $S_f^2 \cdot S_s^2$ for Model 5. b) Values of $\tau_{\text{int}}$ whenever $\tau_{\text{int}} \geq 0.5$ ns. Residues fitting Model 2 are shown in open squares, while residues fitting Model 5 are shown in filled circles. c) Chemical exchange terms, $R_{\text{ex}}$, from Models 3 and 4.
Figure 3. Chemical exchange parameters, $R_{\text{ex}}$, for OspA at 318 K mapped onto the crystal structure. Surface generated using SwissPDBView. Backbone atoms of residues requiring exchange terms are shown in green, the partially buried charged residues associated with the putative binding site are shown in blue (Arg-139, Lys-189) and red (Glu-160), residues forming the hydrophobic cavity that do not require exchange terms are shown in purple (Ile-243), and residues forming the hydrophobic cavity that do require exchange terms are shown in yellow (Leu-167, Leu-178, Leu-187, Phe-237). $\beta$-strands 16 and 17 (ribbon) and the side chain of Trp-216 (stick) are shown in pink.
Figure 4. Apparent activation energies for chemical exchange plotted vs. residue number. The locations of structural elements are indicated by gray bars. Residues for which $R_{ex} < 0.75 \text{ s}^{-1}$ at 318 K or $\chi^2 > 3$ are not shown since these values may not be reliably determined. 

a) N-terminal region and central sheet (residues 25 – 147)  
b) C-terminal region (residues 148-273). Similar activation energies are clustered in strands 12 – 16, 17 – 21, and the C-terminal helix. Figure generated using PrestoPlot.
**Figure 5.** Proposed mechanism for OspA conformational change. a) Hinge closed conformation of OspA observed in the crystal structure, 1osp. b) Proposed hinge open conformation of OspA created by rotating the $\psi$ torsion angle of Lys-212 by $-25^\circ$, $\phi$ and $\psi$ of Thr-213 by $+6^\circ$ and $-39^\circ$, respectively, and $\chi_1$ and $\chi_2$ of Trp-216 by $-109^\circ$ and $+49^\circ$, respectively. c) Proposed model for stabilized open conformation created by extending the $\beta$-sheet. Color scheme is: blue, partially buried positively charged residues Arg-139 and Lys-189; red, partially buried negatively charged residue Glu-160; green, hydrophobic residues associated with the solvent-inaccessible hydrophobic cavity identified in the crystal structure (Leu-167, Leu-178, Leu-187, Phe-237, Ile-243) and functional binding region identified by mutation (Phe-237, Ile-243). Figure generated using SwissPDBView\textsuperscript{53}. 