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FOR THE RECORD

A solution SAXS study of *Borrelia burgdorferi* OspA, a protein containing a single-layer β-sheet

ZIMEI BU[1], SHOHEI KOIDE,[2] AND DONALD M. ENGELMAN[1]

[1]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511
[2]Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, New York 14642

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Abstract: The crystal structure of a soluble form of *Borrelia burgdorferi* outer surface protein A (OspA) complexed with the Fab fragment of a monoclonal antibody has revealed an unusual structure that has a repetitive antiparallel β topology with a nonglobular, single layer β-sheet connecting the globular N- and C-terminal domains. Earlier NMR studies have shown that the local structure of OspA including the single layer β-sheet is similar to the crystal structure. Here we report a small angle X-ray scattering (SAXS) study of the global conformation of OspA in solution. The radius of gyration (R_g) and the length distribution function ($P(r)$) of OspA measured by SAXS in solution are nearly identical to the calculated ones from the crystal structure, respectively. The NMR and SAXS experiments complement each other to show that OspA including the central single-layer β-sheet is a stable structure in solution, and that the OspA crystal structure represents the predominant solution conformation of the protein.

Keywords: outer surface protein; protein folding; small angle X-ray scattering; solution conformation

A continuing challenge in current structural biology is to identify the relative importance of different factors in protein stability and folding. These factors include the hydrophobic effect, hydrogen bonding, packing, and electrostatic interactions. Studies from a variety of soluble proteins have established that the formation of a hydrophobic core that removes hydrophobic surface from contact with water is a major force for protein folding (Kauzmann, 1959; Tanford, 1980; Dill, 1990). However, the crystal structure of a soluble form of outer surface protein A (OspA, an abundant immunogenic lipoprotein of Lyme disease spirochete *Borrelia burgdorferi*) complexed with the Fab fragment of a monoclonal antibody has revealed an unusual structure (Li et al., 1997). OspA has a repetitive antiparallel β topology with a nonglobular, single layer β-sheet connecting the globular N- and C-terminal domains (see Fig. 1A). Interestingly, this central β-sheet consists largely of polar amino acids and it is solvent-exposed on both sides. Such a solvent-exposed, single-layer β-sheet has never been found previously; most antiparallel β-sheets observed in protein structures are amphipathic, with the hydrophobic face buried in the interior hydrophobic core. Considering the well-accepted principles that the formation of the hydrophobic core is the major driving force for protein folding, one may ask if the central β-sheet in OspA is only stabilized in the crystal structure due to antibody binding and/or lattice packing. One might expect that the OspA molecule is bent along the single-layer β-sheet in the free form. To elucidate the molecular basis for the high conformational stability of the unique single-layer β-sheet, it is essential to first establish whether the crystal structure represents the predominant conformation of OspA in solution and thus can be used as a reliable model for the solution structure.

In an earlier study, NMR chemical shift analysis suggested that the local structure of OspA in solution, including the central β-sheet, is similar to the crystal structure (Pham et al., 1998). Amide hydrogen exchange measurements revealed that the central β-sheet is highly stable (Pham et al., 1998). Further, ^{15}N dynamics measurements showed that the tumbling rates of individual amide H-N vectors due to the global tumbling of the protein are consistent with the whole protein being a rigid entity (Pham & Koide, 1998). However, large uncertainties associated with the dynamics measurements precluded drawing a definitive conclusion as to whether the overall shape of OspA in solution is nearly identical to the crystal structure. A global structural measurement is needed to strengthen the case.

Here we report a small angle X-ray scattering study on the OspA in solution. SAXS measures the molecular weight, size, and shape of a molecule, and gives information about the global conformation of a protein in solution. From the SAXS measurements, the radius of gyration R_g, the length distribution function $P(r)$, and the maximum dimension D_{max} provide a direct comparison with the crystal structure. Our SAXS results demonstrated that the R_g and the $P(r)$ of OspA measured by SAXS in solution are identical to the calculated ones from the crystal structure, respectively. The NMR and SAXS experiments complement with each other to show that OspA as well as the central single-layer β-sheet is a stable structure in solution.

Reprint requests to: Shohei Koide, Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, New York 14642; e-mail: Shohei_Koide@urmc.rochester.edu.

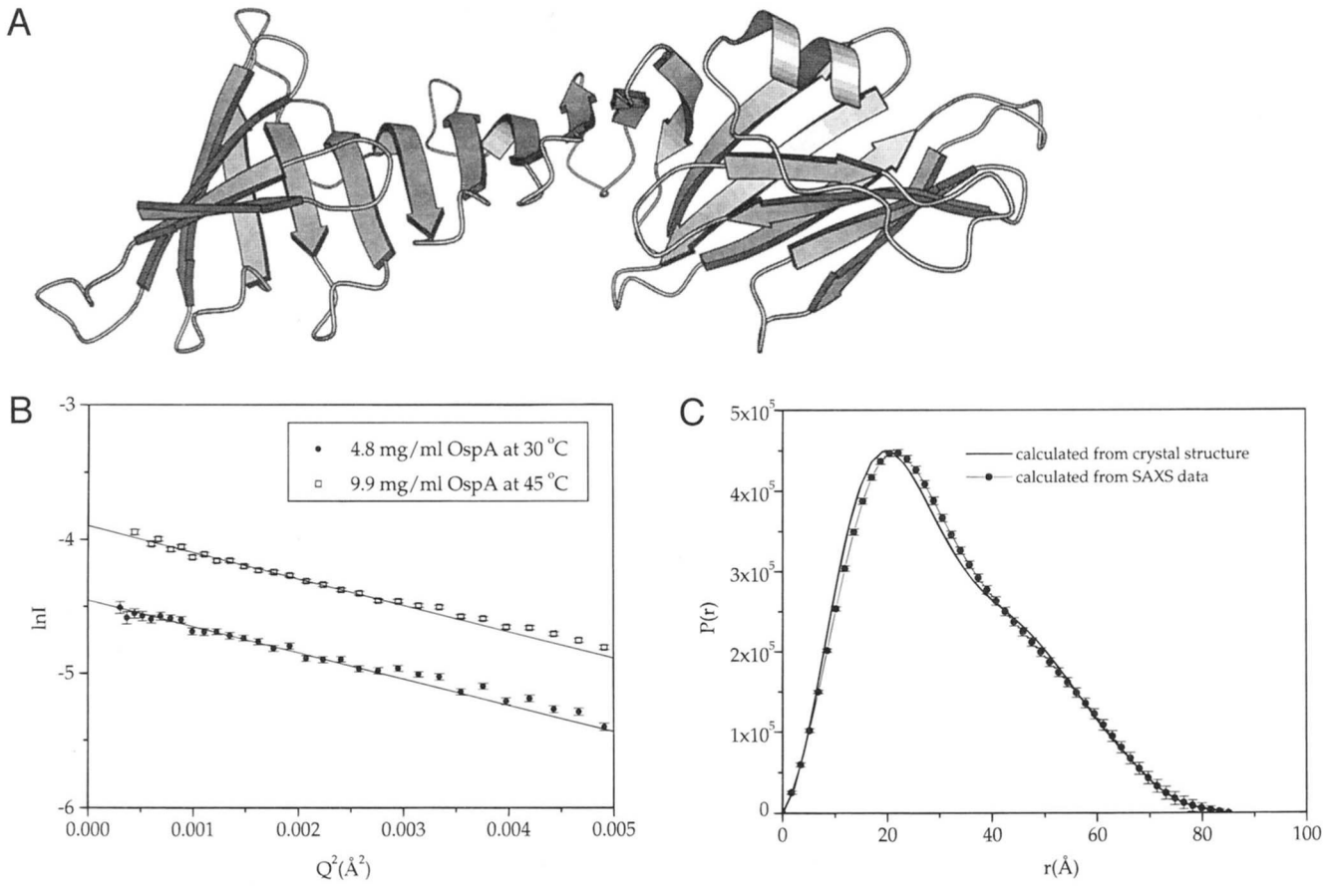

Fig. 1. A: Schematic view of the crystal structure of OspA (PDB entry code: 1OSP, Brookhaven Protein Data Bank). The Fab fragment of an antibody cocrystallized with OspA is not shown here. The central β-sheet is single-layered and solvent-exposed on both sides of the sheet. The figure was made with the program MOLSCRIPT (Kraulis, 1991). **B:** Guinier plots of OspA (•) 4.8 mg/mL at 30 °C and (□) 9.9 mg/mL at 45 °C. **C:** $P(r)$ functions of OspA at 30 °C. Line is calculated from the crystal structure from the Brookhaven Protein Data Bank; (—•—) is generated from the scattering curve. Although there is a small difference of about 1 Å between the SAXS $P(r)$ function and the one from the crystal structure around the length maximum distribution peak, the measured radius of gyration and D_{max} and the calculated ones are identical within experimental error. The small difference could be attributed to the slight fluctuations of the protein molecule, or due to an error in buffer background subtraction in the SAXS experiment.

Results and discussion: The R_g was obtained from the Guinier approximation:

$$\ln I(Q) = \ln I(0) - \tfrac{1}{3}R_g^2Q^2$$

by linear least-squares fitting in the $QR_g \leq 1$ region, where $I(0)$ is the forward scattering intensity and related to the molecular weight of the protein. Two protein concentrations were used: one was at 9.9 mg/mL and the other was at 4.8 mg/mL. No concentration dependent effect was observed by examining the R_g values at these two concentrations, indicating that the protein concentrations were low enough and the intermolecular interaction effects could be ignored.

Figure 1B is the Guinier plot of OspA measured at two concentrations at 30 and 45 °C, respectively. The radii of gyration were 24.3 ± 0.9 Å at 30 °C and 24.4 ± 0.7 Å at 45 °C. The measured R_g's agreed with, within error, the calculated R_g value of 24.7 Å from the PDB file. This suggests that the size of OspA in solution was the same as the one in the crystal structure. Figure 1C is the $P(r)$ function calculated from the solution scattering data and the crystal structure. The $P(r)$ function can provide a direct view of the global conformation of a protein. The shape of the $P(r)$ functions matched with each other very well, indicating that the overall conformation of OspA in solution was similar to that in the crystal. The D_{max} = 85 Å in solution is also identical to the calculated one from the crystal structure, 85 Å, confirming the previous NMR dynamics measurement results that OspA molecule is as rigid as the crystal structure and the central single-layer β-sheet does not have large bending and stretching conformational movements (Pham et al., 1998). Analysis of the crystal structure suggests that cross-strand Glu-Lys pairs in the central β-sheet may provide stabilizing electrostatic interactions (Li et al., 1997; Pham et al., 1998). The central β-sheet is identified to have an abnormally increased thickness between the surface and the β-sheet backbone to bury a comparative amount of nonpolar surface as in the hydrophobic core of a small protein (Pham et al., 1998).

The SAXS study presented here shows that the global structure of OspA in solution is nearly identical to the crystal structure. Taking together with the previous NMR results, we have demonstrated that, despite the unusual single layered β-sheet in the center

of the protein, OspA is stable and rigid in solution, and that the crystal structure represents the predominant conformation of OspA in solution. This study indicates that the combination of SAXS, which gives information about the global structure, and NMR, which gives information about the local structure, provides a powerful means to comprehensively define the solution conformation of a macromolecule. In structure calculation based on NMR data, there are very few experimental restraints for the global conformation, except for data from the ^{15}N dynamics measurements (Tjandra et al., 1997). Thus, SAXS may provide valuable restraints for the global structure for solution determination of macromolecules.

Materials and methods: A truncated form of OspA lacks the first 17 amino acids on the N-terminus and was expressed and purified as described before (Pham & Koide, 1998). The protein was exchanged into 10 mM sodium phosphate, 50 mM NaCl and 50 μM EDTA, pH 6.0 buffer with a small Sephadex G25 column (Pharmacia NAP-5) after repeatedly concentrating and diluting with the buffer with a Centricon-10 (Amicon, Beverly, Massachusetts). Protein concentrations were determined by UV absorption at 280 nM; ϵ_{280} of the protein is 0.284 cm^{-1} mL mg^{-1} (Dunn et al., 1990).

The SAXS instrument and SAXS experiment method were as described before (Bu et al., 1998). The X-ray beam was circularly collimated. No desmearing of the scattering data was necessary by comparing the scattering data of a protein standard, bovine serum albumin, with and without taking account of the beam profile. The sample-to-detector distance was 2.33 m. This enabled an effective Q range of 0.014 to 0.30 $Å^{-1}$ to be measured where $Q = 4\pi \sin\theta/\lambda$ is the magnitude of the scattering vector, 2θ is the scattering angle, and λ = 1.54 Å is the wavelength of the X-rays. The $P(r)$ functions were generated from the scattering data by the GNOM program (Semenyuk & Svergun, 1991). The scattering curve of OspA in the OspA-Fab complex was computed from the crystal structure (PDB ID code: 1OSP, Brookhaven Protein Data Bank) using the CRYSOL program (Svergun et al., 1995). Measurements were done at 30 and 45 °C, respectively.

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