8-11 Solution NMR Structure and X-ray Absorption Analysis of the C-terminal Zinc Binding Domain of the SecA ATPase

SecA is the ATPase subunit of bacterial preprotein translocase. The extreme C-terminus of SecA from *E. coli* harbours a highly conserved Zn^{2+}-binding domain, "SecA-ZBD". This 22 residue sequence, when bound to Zn^{2+}, is important for interactions with the translocation-specific chaperone, SecB, and with acidic phospholipids. A phylogenetic analysis of SecA molecules coded in bacterial genomes indicates that the SecA-ZBD is present in organisms that do not have a gene for SecB, indicating that this domain may have additional functions. The ZBD is attached to the main body of the SecA enzyme by a non-conserved and flexible linker; the linker and ZBD are not present in the crystal structure of SecA from *B. subtilis*, and they must be removed prior to crystallization of *E. coli* SecA. To obtain an atomic-resolution structure for the SecA ZBD, we used a combination of NMR spectroscopy and X-ray scattering.

The sequence of the 22 residue peptide used for these studies was: Ac-KVGRNDPCPCGSGKKYKQCH-GRLQ. We used two-dimensional proton NMR to solve the structure of the SecA-ZBD. From this work, we learned that the Zn^{2+}-bound SecA-ZBD forms a tightly folded and stable structure which is stabilized primarily through peptide-Zn^{2+} interactions, as well as the formation of a small hydrophobic core consisting of Val2 and Tyr16.

The NMR experiments did not provide us with any direct information on the location of the Zn^{2+} ion. We did model a Zn^{2+} ion into the structure, but the coordination geometry and bond distances between Zn^{2+} and the side chains of Cys8, Cys10, Cys19, and His20, appeared to be distorted. To obtain detailed information on the interactions between the Zn^{2+} ion and the peptide, EXAFS experiments were conducted at BL-12C. The absence of a peak at 9663 eV in the XANES spectrum of the SecA-ZBD (Fig. 23(A)) was consistent with 4-coordination of the Zn^{2+} ion. The EXAFS spectrum and corresponding Fourier transform (Fig. 23(B)) were used to model the four Zn^{2+} ligands as three sulphur atoms, with an average Zn^{2+}-S distance of 2.30 Å, and one nitrogen atom at a distance of 2.03 Å. Incorporation of the constraints into our structure calculation allowed us to include the Zn^{2+} atom in the NMR-derived structure (Fig. 24).

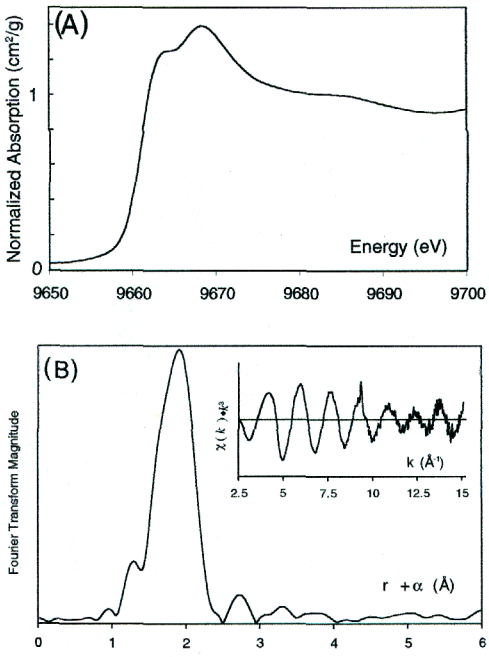

Figure 23
X-ray absorption analysis of the SecA-ZBD. (A) Normalized XANES spectrum (B) k^3-weighted Zn^{2+} K-edge EXAFS spectrum and corresponding Fourier Transform (inset). Reproduced from *Biochemistry* 2004, 43:9361-9371. Copyright 2004 Am. Chem. Soc.

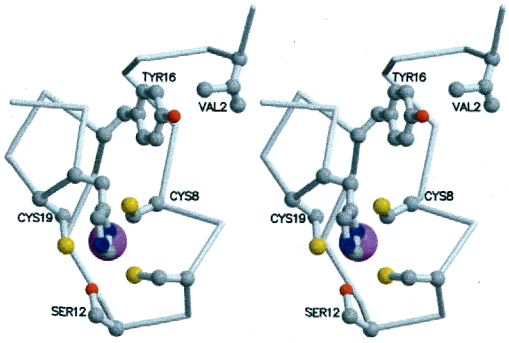

Figure 24
Stereoview of the Structure of the Zn^{2+}-bound SecA-ZBD using combined NMR and EXAFS data. Reproduced from *Biochemistry* 2004, 43:9361-9371. Copyright 2004 Am. Chem. Soc.

Some of the noteworthy features of the structure include the fact that two of the Zn^{2+} ligands, Cys19 and His20, are adjacent to each other. This is extremely uncommon for Zn^{2+} binding proteins, probably because of the relatively high energy required for proper positioning of the two side chains. In fact, Cys19 is the only residue in the structure whose main chain conformation falls in a disallowed region of the Ramachandran plot ($\phi = -150°$, $\psi = -79°$). Another interesting feature is the presence of Ser12 which makes important "second sphere" interactions with the Zn^{2+}-binding residues. Ser12 is strictly conserved among all known SecA-ZBD sequences, and our NMR data show that the Oγ proton is in slow exchange with solvent, and there are NOEs present between this proton and a number of other protons in its vicinity. Ser12 appears to be important because it makes a very strong hydrogen bond with Cys19.

This type of Zn^{2+} binding motif is not present in the genomes of eukaryotes; however, it is present in a number of proteins of unknown function from prokaryotes. We are investigating whether these proteins are involved in secretion, or if they have an unrelated function.

B. Dempsey[1], F. Jalilehvand[2], G. S. Shaw[1], and B. Shilton[1] ([1]*Univ. of Western Ontario*, [2]*Univ. of Calgary*)

References

[1] B. R. Dempsey, M. Wrona, J. M. Moulin, G. B. Gloor, F. Jalilehvand, G. Lajoie, G. S. Shaw and B. H. Shilton, *Biochemistry*, **43** (2004) 9361.