Structural and Functional Studies on Mip (Macrophage infectivity potentiator protein) from *Chlamydia*

Aida Baharuddin, Manuel Kaulich, Jörg Deiwick and Rolf Hilgenfeld

Institute of Biochemistry, University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany

Chlamydia spp. are obligate intracellular bacteria that act as human pathogens, causing various forms of chlamydiasis. One of the virulence proteins essential for survival of the bacteria inside human macrophages is the macrophage infectivity potentiator, Mip. Mip proteins have been identified in several intracellular parasites, such as *Chlamydia trachomatis* [1]*, Legionella pneumophila* [2], and *trypanosoma* cruzi [3].

In order to shed light onto the complex mechanisms of intracellular infection, we are studying Mip proteins and other virulence proteins of *Chlamydia spp*. and *Legionella spp*.. We have already determined the crystal structure of *Legionella pneumophila* Mip [4]. The protein is a homodimer, with each polypeptide chain forming two domains that are connected by an extremely long (66 Å), isolated α-helix. The N-terminal domain is completely α-helical and is responsible for the dimerization of the molecule, through the formation of an intermolecular four-helix bundle and what we call a methionine zipper [4]. The C-terminal domain resembles the human FK506-binding protein, and indeed, *Legionella* Mip exhibits a peptidyl-prolyl *cis/trans* isomerase activity that is inhibited by FK506 ("tacrolimus"). We have also determined the structure of the Mip complex with FK506 and located the drug in the binding site for the substrate peptide [4].

Legionella Mip is a very basic protein, with a pI of above 9. In contrast, the orthologous protein from *Chlamydia spp*. is acidic. This property appears to be connected to the location of the protein and its function. In addition to the expected peptidyl-prolyl *cis/trans* isomerase activity, we found a general chaperone function for *Chlamydia* Mip but not for *Legionella* Mip [5]. Bacterial chaperones involved in effector injection from bacteria into a host cell, i.e. *via* type III or IV secretion systems, are usually acidic, homodimeric proteins, often with a C-terminal α-helix.

We are working on the structure determination of the Mip proteins from *Chlamydia pneumoniae* and *Chlamydia trachomatis*. Various crystal forms were tested at beamline X13, but the experiments showed that the diffraction quality of the crystals will have to be improved to allow structure elucidation.

References

[1] A.G. Lundemose, D.A. Rouch, S. Birkelund, G. Christiansen, J.H. Pearce, Mol. Microbiol. 6, 2539-2548 (1992)

[2] N.C. Engleberg, C. Carter, D.R. Weber, N.P. Cianciotto, B.I. Eisenstein, Infect. Immun. 57, 1263-1270 (1989)

[3] P.J. Pereira, M.C. Vega, E. Gonzalez-Rey, R. Fernandez-Carazo, S. Macedo-Ribeiro, F.X. Gomis-Ruth, A. Gonzalez and M. Coll, EMBO Rep. 3, 88-94 (2002)

[4] A. Riboldi-Tunnicliffe, B. König, S. Jessen, M.S. Weiss, J. Rahfeld, J. Hacker, G. Fischer and R. Hilgenfeld, Nature Struct. Biol. 8, 779-783 (2001)

[5] A. Vogel, PhD thesis, University of Jena (2005)