Structure and function of the *Borrelia burgdorferi* porins, P13 and P66

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Abstract

*Borrelia burgdorferi* is a helically shaped bacterium with a parasitic life style, always present within a host. The bacterium is transmitted by means of infected ticks to small rodents or birds that usually serve as reservoirs. Infected ticks can bite humans and there the bacterium cause Lyme disease. Early infection involves the skin and in many cases a typical skin rash called erytema migrans, can be seen at the site of the tick bite. If left untreated the disease can cause impairments of various organs such as joints, heart and nervous system.

Porins are water-filled channels situated in the outer membrane of bacteria. These proteins are important in the sieving mechanism of the outer membrane, allowing important compounds to pass into the bacterial cell, while excluding harmful substances. *Borrelia* spp. are limited in metabolic and synthetic pathways and are therefore highly dependent on their surrounding to supply them with important compounds. Because of this, porins are believed to be very important for *Borrelia* spp. Except a role in nutrient acquisition, porins can function as adhesins and in bacterial adaptation to new environments with variations in osmotic pressure.

P13 and P66 are two integral outer membrane proteins in *B. burgdorferi*, previously seen to have porin activities. In addition to the porin activity, P66 is also an adhesin, binding integrins present on for instance epithelial cells in the human body. In this thesis structural characterisations and functional studies of P13 and P66 were assessed. In the Black lipid bilayer assay, the pore forming activity of P13 was shown to be much smaller than previously thought, exhibiting activity at 0.6 nS with a channel size of 1.3 nm. Initial Nanodisc experiments displayed a pore size of 1.3 nm, confirming the Black lipid bilayer results. The 300 kDa protein complex formed by P13 contained sole P13 monomers. P66 forms pores with a single channel conductance of 11 nS and a channel size of 1.9 nm. The porin assembles into a large protein complex of 420 kDa in the outer membrane, containing exclusively P66 monomers. The integrin-binding function of P66 was found to be important for efficient bacterial dissemination in the murine host but was not essential for *B. burgdorferi* infectivity. Neither P13 nor P66 had an active role in osmotic stress adaptation. Instead, two *p13* paralogs were up-regulated at the transcript level in *B. burgdorferi* cultured under glycerol-induced osmotic stress.

Keywords

*Borrelia*, P13, P66, porin, protein complex, dissemination, osmotic stress