Characterization of proteins encoded by *hmu* operon from *Porphyromonas gingivalis* and other bacteria.

The aim of this PhD thesis was to characterize proteins encoded by the *hmu* operon in *Porphyromonas gingivalis* and other bacteria. Specific aims included further characterization of the HmuY protein in regard to heme acquisition by *P. gingivalis* and initiation of studies on mechanisms of heme acquisition by selected HmuY homologs from other periodontopathogens, namely *Prevotella intermedia* (PinA and PinO proteins) and *Tannerella forsythia* (Tfo protein).

P. gingivalis HmuY binds not only heme, but also its derivatives, Fe(III)MPIX (mesoheme) and Fe(III)DPIX (deuteroheme), as well as heme with iron replaced by other metals: Ga(III)PPIX, Mn(III)PPIX, Cu(II)PPIX, Co(III)PPIX, Zn(II)PPIX, Ni(II)PPIX. However, metalloporphyrins with other metals are bound in a different manner compared to heme, mesoheme, and deuteroheme. Our results indicate that only Co(III)PPIX is coordinated by two histidines while all the other metalloporphyrins interacts with HmuY protein through either H134 or H136. Stability analysis of protein variants, in which tryptophan residues were replaced by alanine or tyrosine residues, demonstrated changes in tertiary structure of the protein, resulting from heme binding.

In contrast to *P. gingivalis* and *T. forsythia,* bacteria possessing one copy of the gene encoding the protein (HmuY and Tfo, respectively), *P. intermedia* 17 possesses two genes encoding HmuY homologs (PinA and PinO). HmuY homolog has not been identified in *T. denticola*, the third „red complex" member. In addition, nucleotide and amino acid sequences of Tfo were corrected. Important differences have been found between examined proteins in amino acid sequences and predicted three dimensional protein structures. Moreover, examined HmuY homologs lack two histidine residues involved in heme iron coordination by *P. gingivalis* HmuY. Examined HmuY homologs bind heme, but in a different manner compared to *P. gingivalis* HmuY. In contrast to HmuY protein, examined homologs are not able to acquire heme bound to host hemoproteins. Interestingly, *P. gingivalis* HmuY not only acquires heme present in host hemoproteins, but also that bound to PinA, and PinO. Preliminary analyses of three dimensional protein structures resulted in satisfactory crystals of HmuY with complexed heme, as well as in spherulites of PinA, but only in case of the protein complexed with heme.

It is likely that in host environment, HmuY homologs produced by other periodontopathogens participate in heme acquisition. However, *P. gingivalis* HmuY may take advantage of heme acquisition mechanisms of other bacteria, present in multibacterial oral biofilm to gain basic nutritional components, such as iron and PPIX and to increase its own virulence.