Abstract

Bacterial protein WrbA from *E. coli* is the founding member of a new family of FMN-dependent NAD(P)H oxidoreductases, forming a functional and structural bridge between bacterial flavodoxin and certain mammalian NAD(P)H:quinone oxidoreductase. For these reasons, protein WrbA is recently intensively studied using various analytical and computing methods. Protein WrbA participates in the protection of cells against oxidative stress, but precise function of the protein WrbA *in vivo* is still unknown. Protein WrbA forms multimers in solutions. In μM concentrations and at low temperature (4 °C) the protein is in the form of a dimer, with increasing temperature becomes tetrameric. Available three-dimensional crystal structure contains the information about the tetrameric form of the protein, the dimeric form has not been structurally characterized.

This thesis was focused on the study of the dynamic behavior of protein WrbA in solution using methods of hydrogen-deuterium exchange and chemical cross-linking followed by mass spectrometric analysis with high resolution (FT-ICR). Behavior of the protein was monitored according to the presence of cofactor FMN. Effect of temperature and protein concentration was also studied. Hydrogen-deuterium exchange provided information about solvent accessibility and dynamics of the entire protein sequence. Cofactor stabilizing effect on the structure of the protein tetramer was described, however, very flexible regions of the amino acid sequences were also found across all experimental conditions. Flexible parts of the sequence were confirmed by chemical cross-linking. Interface of dimer formation in apoprotein solution was also designed combining data obtained by hydrogen-deuterium exchange and chemical cross-linking. (in Czech)

Key words: mass spectrometry, protein conformation, chemical cross-linking, hydrogen-deuterium exchange, Tryptophan repressor binding protein