Abstrakt EN

Heme containing gas sensor proteins play important role in bacterial physiology in regulating many processes such as cell differentiation, virulence, biofilm formation or intercellular communication. For their structure, typical modular architecture is characteristic where various sensor domains (usually at the N-terminus) regulate the activity of the catalytic or functional domains (usually at the C-terminus). In this dissertation thesis, we focused on three representatives from the group of oxygen sensing proteins, namely histidine kinase \(Af\)GcHK, diguanylate cyclase YddV, phosphodiesterase \(Ec\)DOS and also on protein RR, which is the interaction partner of \(Af\)GcHK.

The main aim of the thesis was to study intra-protein/inter-domain signal transduction in two representatives of heme sensor proteins with a globin fold of the sensor domain (\(Af\)GcHK, YddV) and in one representative with PAS fold of the sensor domain (\(Ec\)DOS). Another objective was to describe inter-protein signal transduction in the two component signaling system \(Af\)GcHK-RR and structurally characterize these two interacting partners. Emphasis was also placed on the study of the interaction between model sensor domains and different signaling molecules and also on function of individual amino acids involved in the binding of these signaling molecules.

Study of intra-protein/inter-domain signal transduction revealed that the active forms of \(Af\)GcHK exhibit very similar \(V_{\text{max}}^{\text{ATP}}\) values, which probably indicates that these states also have similar conformations responsible for the optimum catalytic activity. Inactive states of \(Af\)GcHK exhibited lower \(V_{\text{max}}^{\text{ATP}}\) values and lower affinity for ATP compared to active states. In case of \(Ec\)DOS protein correlation between the activity of its functional domains and flexibility of its sensor PAS domain was observed. Flexibility of sensor domain of the least active form containing \(Fe^{3+}\) heme iron complex was higher than the flexibility of sensor domain of more active forms containing \(Fe^{2+}\) and \(Fe^{2+}-CO\) heme iron complexes.

Study of inter-protein signal transduction between \(Af\)GcHK and RR brought information about mutual interaction interface of these two proteins, which is localized in \(Af\)GcHK’s dimerization domain and a regulatory domain no. 1 of RR protein. Complex \(Af\)GcHK-RR is transient, with a high rate of dissociation, where the \(K_d\) for the complex of dimeric \(Af\)GcHK with one molecule of RR protein is about 18 µM.

Examining the interaction of YddV sensor domain and its mutant forms with different signaling molecules confirmed the importance of Leu65 in stabilizing of these signaling molecules. Leu65 prevents entry of water molecules into the heme distal side. Activating effect of the new signaling molecule \(H_2S\) was demonstrated only in case of \(Ec\)DOS protein, while the activity of \(Af\)GcHK was not affected by the \(H_2S\) treatment.