Abstract

Heme is one of the most important and most studied cofactors that are essential for proper function of many proteins. Heme-containing proteins comprise of a large group of biologically important molecules that are involved in many physiological processes. The presented dissertation is focused on two groups of heme sensor proteins, namely prokaryotic heme-based gas sensors and eukaryotic heme-responsive sensors. Heme-based gas sensors play an important role in regulation of many bacterial processes and consist usually of two domains, a sensor domain and a functional domain. The dissertation thesis aims at the study of two model bacterial heme-based gas sensors, histidine kinase *Af*GcHK and diguanylate cyclase YddV, in order to elucidate their mechanism of interdomain signal transduction. Using X-ray crystallography and hydrogen-deuterium exchange coupled to mass spectrometry approaches, significant differences in the structure of the *Af*GcHK protein between the active and inactive forms were described. The signal detection by the *Af*GcHK sensor domain affects the structural properties of the protein, and these conformational changes then have indirect impact on the enzyme activity of the functional domain. Further, the dissertation pays more attention to the effect of a sensor domain dimerization interface arrangement on a signal transduction. When the dimerization of the sensor domain is disrupted, the ability of the subsequent signal transduction is affected, and the protein thus loses its enzyme activity. A detailed kinetic analysis helped us to reveal how changes in the sensor domain of the protein affect its catalytic activity in the case of the second studied protein, the diguanylate cyclase YddV. This study showed that the catalytic activity depends significantly on the redox and ligand state of the heme iron. Analysis of oligomeric states then exposed that YddV forms dimers in solution and its inactive mutant form H98A tends to form octamers. These results suggest that the oligomeric state is crucial for optimal protein function, as in the case of *Af*GcHK. The dissertation thesis contains also a comprehensive review which focuses on eukaryotic heme-based sensors and summarizes the newly discovered role of heme as a signal molecule.