Signal sensing and signal transduction in heme sensor proteins

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The most popular biological function of heme is to act as a prosthetic group in hemeproteins that show a variety of functions, including oxygen storage and transport, electron transfer, redox catalysis and sensor of gas molecules. Besides acting as a prosthetic group in protein matrix, it has become apparent that a free heme molecule can act as a physiological effector of several proteins, including transcriptional regulators, heme-regulated eIF2α kinase, and sensor kinases in two-component signal transduction systems. Reversible heme binding regulates the physiological function of these proteins. Though the research on these proteins have shown a new physiological function of heme as a signaling molecule, detail molecular mechanisms by which heme regulates the biological functions of these proteins remain to be elucidated mainly because the three-dimensional structures of these regulatory proteins have not been solved yet.

Numerous lactic acid bacteria including *Lactococcus lactis* acquires heme molecules as an exogenous source of heme to establish an aerobic respiratory chain. As free heme molecules are toxic for cells, cellular concentrations of heme should control strictly. *L. lactis* controls cellular heme concentrations by operating a heme efflux system. The expression of the heme efflux system is regulated by a heme-sensing transcriptional regulator HrtR (heme related transporter regulator). In this work, we have determined the crystal structures of apo-HrtR/DNA complex, apo-HrtR, and holo-HrtR at a resolution of 2.0, 2.8, and 1.9 Å, respectively, to elucidate the structure and function relationships of HrtR.

HrtR is a homo-dimer in the both of apo- and holo-forms. HrtR monomer consists of the N-terminal DNA-binding domain and the C-terminal heme binding domain that binds one heme molecule. Titration of apo-HrtR with heme revealed that apo-HrtR takes up 1 mol equivalent of heme to form an 1:1 complex that shows the Soret peak, a and b bands at 413, 562 and 536 nm, respectively, in the ferric form. Reduction of holo-HrtR changed the Soret, a and b peak to 425, 560, and 530 nm, respectively. These spectra are similar to those of hemeproteins with bis-His axial ligands, suggesting that the heme in holo-HrtR is coordinated by two histidines. The crystal structure of holo-HrtR described below showed that it is the case.

To obtain structural insights into the heme-responsive regulatory mechanisms of HrtR function, we compared the crystal structures of apo-HrtR/DNA complex and holo-HrtR. Global fold of HrtR is similar to that of TetR family transcriptional regulators. HrtR is the first example of heme-sensing TetR family transcriptional regulator. A change in the relative orientation of the DNA-binding domain is induced upon heme-binding, which results in the regulation of DNA-binding activity of HrtR.

We have found that apo-HrtR can bind the target DNA, but holo-HrtR can not. DNA-binding affinity of apo-HrtR is determined to be Kd = 0.2 nM by fluorescence anisotropy measurements. These results indicate that heme molecule acts as a physiological effector of HrtR to regulate its DNA-binding activity.

The apo-HrtR/DNA complex structure reveals how the apo-HrtR dimer binds target DNA. The helix-turn-helix motif of each protomer contacts two consecutive DNA major grooves, and α2 and α3 helices in the helix-turn-helix motifs interact with the target DNA. Arg46 (εNH) forms a hydrogen bond with a base (N7 of G11 (guanine 11)), which is the sole hydrogen bond involving the bases of DNA. The guanidium group of Arg46 also forms hydrogen bonds with the phosphate backbone of T10 (thymine10) and Tyr50. There are also hydrogen bonds between the imidazole group of His37 and the phosphate group of G9 (guanine 9), between the NH group of Ile35 and the phosphate group of T10, between the backbone NH group of Met36 and the phosphate group of T10, and between the OH group of Tyr50 and the phosphate group of G11. In addition to these hydrogen bonds, a CH-π interaction should be present between the methyl group of T12 (thymine 12) and Tyr50. The methyl group of T12 is oriented perpendicularly to the phenyl ring of Tyr50, and the distance is 3.8 Å between the carbon atom of the methyl group of T12 and the Cg of Tyr50, which are reasonable conditions for a CH-π interaction.

The crystal structure of holo-HrtR revealed that the heme is accommodated in a large cavity that is open to solvent, with His72 and His149 as the axial ligands to form a 6-coordinated heme. The α4, α5, α8, and α9 helices compose a hydrophobic heme pocket in the cavity, where the heme is surrounded by hydrophobic residues Ile71, Phe75, Phe76, Leu95, Leu99, Phe112, Val148, and Val152. These hydrophobic interactions along with the axial ligation of His72 and His149 will be responsible for a high heme-binding affinity of HrtR.

While a loop from Pro125 to Glu135 is located over the edge of the opening of the heme-binding cavity on the protein surface in holo-HrtR, the electron density of this loop was not observed in apo-HrtR, probably due to disorder, suggesting flexibility of the loop from Pro125 to Glu135. This flexible loop may change its conformation over the entrance of the heme-binding cavity, by which the heme-binding affinity would be regulated.

A comparison of the apo-HrtR/DNA complex and holo-HrtR structures revealed that heme-binding triggers a coil-to-helix transition at the α4a-α4b region. In apo-HrtR, a loop (residues 68-71) intervenes between α4a and α4b helices. Glu70 in the middle of this intervening loop forms a hydrogen bond with Trp123 in apo-HrtR. Upon heme-binding, a coil-to-helix transition occurs in this intervening loop, which results in the formation of a long α4 helix in holo-HrtR. As the location of Glu70 is largely altered by this coil-to-helix transition, the hydrogen bond between Glu70 and Trp123 is lost in holo-HrtR.