Development of Artificial Enzymes Based on the Myoglobin Scaffold

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Heme proteins play key roles in oxidative reactions, oxygen storage, and electron transfer in living organisms. Despite the variety of these functions, all of the heme proteins commonly contain a heme cofactor at the active site. Therefore, amino acid residues surrounding the heme should greatly contribute to the expression of various functions. This idea strongly suggests that an artificial heme protein with a novel function can be created by substituting amino acid residues around heme. In this study, we have attempted to develop heme proteins that have peroxidase activity, based on the myoglobin (Mb) scaffold.

Mb is one of the heme proteins most extensively studied. Mb's function is mainly oxygen storage, although it shows weak peroxidase activity. Mb possesses a heme pocket at the active site that is advantageous to the binding of exogenous small ligands such as O_2, CO, and CN^-. In order to improve access of exogenous ligands to the active site, we created Mb H64V/V68H/H93A/H97F (VHAF) and H64V/V68H/H93A/H97A (VHAA) mutants. We determined crystal structure of these quadruple mutants and found that the heme is exposed to the solvent in the mutants. However, they still retained globin-fold, consisting of unique 8-helical structure. Furthermore, the VHAA showed peroxidase activity with 6-fold larger V_{max} and 45-fold smaller K_m than wild-type Mb. These results suggest that it is possible to create a novel artificial protein successfully by utilizing globin-fold. Further mutations may allow development of a more functionalized protein, which is a nano-machine with high potential.