Coordinated Design of Cofactor and Active Site Structures in Development of New Protein Catalysts

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New methods for the preparation of artificial metalloenzymes are important for the construction of new biocatalysts and biomaterials. We have reported a novel method for the preparation of artificial metalloenzymes, i.e., removal of the heme prosthetic group from myoglobin (Mb) followed by the insertion of symmetric Cr^{III} Schiff-base complexes, which are known as oxidation catalysts in organic solvents, into the empty cavity of apo-Mb (M. Ohashi et al, *Angew Chem. Int. Ed.* 2003, **43**, 1005-1008), but the low reactivity still remained because there was little structural information. We have now made coordinated design of cofactor and active site structures of M^{III}(Schiff-base)•apo-Mbs (M=Cr and Mn) based on the crystal structures (Figure 1). The structures suggest that the position of the metal complex is determined by non-covalent interaction between the ligand and the surrounding peptides, especially, specific interactions of Ile107 with 3 and 3'-methyl groups of the ligands. Thus, replacement of the methyl groups with larger substituents is expected to control the penetration depth of the Schiff-base ligand in the active site. If this is the case, we would be able to controle the enantioselectivity in the sulfoxidation of thioanisole. In fact, the yield of *S*-methyl phenyl sulfoxide is decreased as the substituent size at the position 3 and 3' are larger and *R*-sulfoxide was eventually obtained when R is a propyl group. This is the first example of the enantioselective enzymatic reaction by the regulation of the position of the inserted synthetic metal complex in the active site.

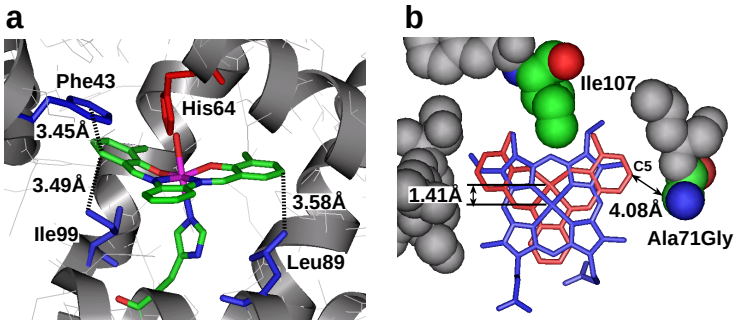

Figure 1. The active site structure of Mn•**1**•apo-A71GMb: side (**a**) and top (**b**) views. Structure of heme of met-Mb is superimposed on Mn•**1**•apo-A71GMb in **b**.