Bioinorganic Chemistry

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An Engineered Metalloprotein as a Functional and Structural Bioinorganic Model System

Takafumi Ueno*

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Despite the rapid advances which have occurred in structural biology and computational chemistry, the rational design of functional protein structures continues to be one of the most challenging objectives in the field of protein engineering involving synthetic model compounds and de novo peptides.[1] The engineering of active sites is more complicated for metalloenzymes than for non-metalloenzymes because individual metal coordination geometries and oxidation states must be taken into account to ensure the desired function.[2] Although elegant de novo designs of heme and non-heme proteins with symmetrical coordination centers have been reported recently,[3,4] metalloenzymes with asymmetrical coordination centers have rarely been mimicked, even through the use of model compounds. Lu and co-workers recently described the rational design of a model metalloprotein containing a sophisticated metal center with the structural and functional properties of a native metalloenzyme.[5] The use of a metalloprotein as a molecular template for mechanistic investigations of reactions catalyzed by other metalloenzymes is expected to be a powerful approach in the field of bioinorganic chemistry.

The target protein of this research was nitric oxide reductase (NOR), which promotes the two-electron reduction of NO to N_2O in a bacterial denitrification system.[6] Despite the lack of a three-dimensional structure, spectroscopic and genetic investigations of native NORs have shown that, structurally, NOR is most closely related to heme–copper oxidases (HCOs). One exception is that NOR contains a unique $Fe(His)_3$ non-heme iron active site instead of the common $Cu(His)_3$ center of the HCOs.[6,7] Previous genetic investigations of NOR indicated the existence at the active center of an invariant glutamate residue, which is not conserved in the HCO active centers.[8,9] This invariant glutamate residue is crucial for enzymatic activity. Model complexes of NOR have been synthesized;[10,11] however, the role of the glutamate has not yet been proven, as a more complicated design is needed for the construction of the asymmetric coordination sphere. Thus, the modeling of NOR is much more challenging than the preparation of active-site models of typical heme and non-heme metal active centers. To address the problem, Lu and co-workers used myoglobin (Mb), a dioxygen-carrier heme protein, in a semisynthetic approach.

The distal histidine residue (His64) of Mb plays a role in the capture of a dioxygen molecule by providing a hydrogen-bonding interaction.[12] His64 was identified as a candidate for a ligand in a model of a non-heme iron center. A comparison of the structures of Mb and cytochrome *c* oxidase (an HOC) suggested that two residues adjacent to His64 (Leu29 and Phe43) could be replaced with histidine residues to construct a $Fe(His)_3$ center (Figure 1). On the basis of this minimized structure, a glutamate residue was introduced at position 68. The triple mutant L29H/F43H/V68E Mb (Fe_BMb) was prepared as an NOR model scaffold. Iron(II)-titration experiments and the high-resolution crystal structure of Fe_BMb containing an Fe^{II} ion (Fe^{II}-Fe_BMb) indicated the likelihood that the conserved glutamate residue helps to stabilize the binding of iron at the active center through monodentate ligation (Figure 2a). Interestingly, the EPR-spectral and electrochemical properties of Fe^{II}-Fe_BMb are almost identical to those of native NOR. The activity of Fe^{II}-Fe_BMb for the reduction of NO was confirmed by gas chromatography/mass spectrometry, although the amount of N_2O formed was very low. The enzymatic mechanism was investigated in detail by comparison with other Mb composites, such as wild-type and Glu-deleted Fe_BMb. Finally, Lu and co-workers concluded that the glutamate residue and the three histidine residues are essential both for iron binding and for NO-reduction activity.

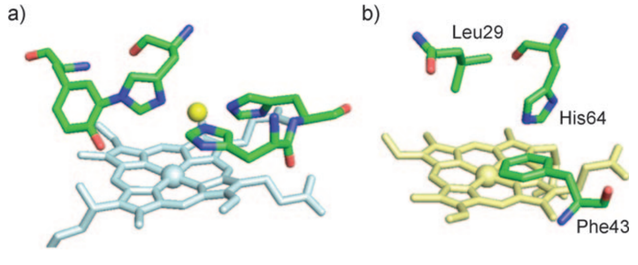

Figure 1. a) The heme–copper center of cytochrome *c* oxidase.[7] b) Residues of the active site of wild-type Mb (PDB ID: 2MBW) to be replaced with histidine residues for the construction of the non-heme site of NOR.

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[*] Prof. T. Ueno
Institute for Integrated Cell-Material Sciences (iCeMS) and
Funai Center, Kyoto University
Katsura, Nishikyo-ku, Kyoto 615-8510 (Japan)
Fax: (+81) 75-383-2812
E-mail: taka@icems.kyoto-u.ac.jp
Homepage: http://www.kitagawa.icems.kyoto-u.ac.jp/index.html

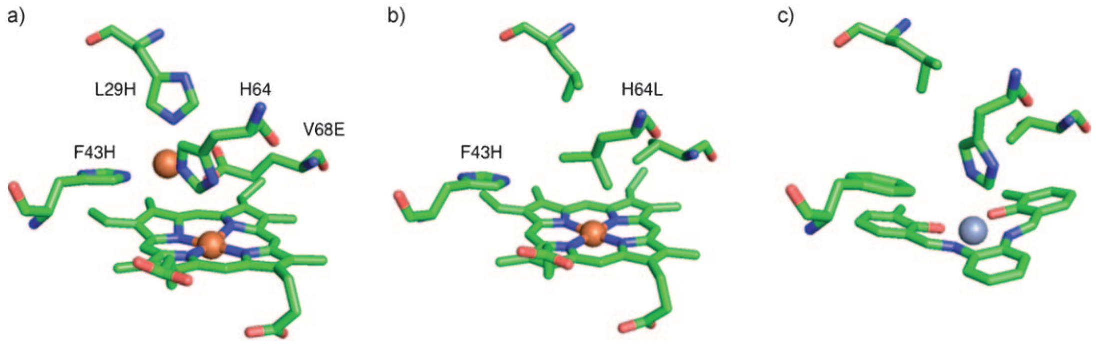

Figure 2. Comparison of a) Fe^{II}-Fe$_B$Mb, b) F43H/H64L Mb, and c) chromium(II)-(*N*,*N'*-bis(3-methylsalicylidene)-1,2-phenylenediamine)-apo-(A71G Mb). The crystal structures are taken from PDB IDs 3K9Z, 1OFK, and 1J3F, respectively.

The combination of site-directed mutagenesis and computational techniques is highlighted in this example of rational protein design; however, a distinctive aspect of this research was recognition of the advantages of using myoglobin as the protein scaffold. As described by the authors, the preparation and crystallization procedures of Mb are very well established. Wild-type Mb and mutants of Mb can be obtained in high yield by using the *Escherichia coli* expression system. Since the first three-dimensional structure of Mb was reported as the first example of a structure of a metalloprotein in 1958, more than 300 structures of Mb and Mb mutants have been determined under various conditions.[13,14] The Mb scaffold can accept different active-center structures and cofactors. For example, Watanabe et al. reported that the original function of Mb as a dioxygen carrier could be manipulated to provide the functions of other hemoproteins, such as peroxidase or peroxygenase activity, by altering the position of His64 in the Mb active site.[15] The reaction mechanisms were discussed on the basis of the crystal structures of the mutants (Figure 2b). Interestingly, the formation of compound I of the Mb mutants can be observed spectroscopically.[16] The heme cofactor of Mb can be replaced with various synthetic cofactors, such as Schiff base complexes with different metal ions (Figure 2c).[17,18] The crystal structures show very few structural deviations from the entire structure of wild-type Mb. Thus, the Mb scaffold is perfectly adapted to the construction of an additional metal-binding site immediately above the heme group. This approach provides a convenient option for the synthesis of metal ligands with complex structures. Lu and co-workers also reported the design of a copper-containing active center similar to that of cytochrome *c* oxidase by engineering of the active center of Mb.[19]

Lu and co-workers have thus demonstrated a new strategy for preparing models of natural metalloenzymes which are difficult to obtain in high yield and for which crystal structures have not been determined. It is expected that this method will be useful for the preparation of models of sophisticated functional metal centers that are difficult to prepare by chemical synthesis. The results are eagerly anticipated not only from the standpoint of the quest for fundamental knowledge about enzymatic reactions of native metalloenzymes but also for the development of new biocatalytic applications. This recent development opens exciting directions in the field of bioinorganic chemistry.

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